WOODLAND DEVELOPMENT AND SOIL CARBON AND NITROGEN DYNAMICS AND STORAGE IN A SUBTROPICAL SAVANNA ECOSYSTEM

A Dissertation

by

JULIA DEN-YUE LIAO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2004

Major Subject: Rangeland Ecology and Management

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Approved as to style and content by:

Thomas W. Boutton (Chair of Committee)	James H. Matis (Member)
Frank M. Hons (Member)	Julie D. Jastrow (Member)
David A. Zuberer (Member)	Steven G. Whisenant (Head of Department)

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ABSTRACT

Woodland Development and Soil Carbon and Nitrogen Dynamics and Storage in a Subtropical Savanna Ecosystem. (December 2004)

Julia Den-Yue Liao, B.S., Brigham Young University;

M.S., Brigham Young University

Chair of Advisory Committee: Dr. Thomas W. Boutton

Woody plant invasion of grasslands is prevalent worldwide, but the biogeochemical consequences of this vegetation shift remain largely unquantified. In the Rio Grande Plains, TX, grasslands and savannas dominated by C_4 grasses have undergone succession over the past century to subtropical thorn woodlands dominated by C_3 trees/shrubs. To elucidate mechanisms of soil organic carbon (SOC) and soil total N (STN) storage and dynamics in this ecosystem, I measured the mass and isotopic composition ($\delta^{13}C$, $\delta^{15}N$) of C and N in whole-soil and soil size/density fractions in chronosequences consisting of remnant grasslands (Time 0) and woody plant stands ranging in age from 10-130 years. Rates of SOC and STN storage averaged 10-30 g C m^{-2} yr^{-1} and 1-3 g N m^{-2} yr^{-1}, respectively. These accumulation rates increased soil C and N pools 80-200% following woody encroachment. Soil microbial biomass (SMB-C) also increased after woody invasion. Decreasing C_{mic}/C_{org} and higher qCO_2 in woodlands relative to grasslands suggests that woody litter is of poorer quality than grassland litter. Greater SOC and STN following woody invasion may also be due to increased protection of organic matter by stable soil structure. Soil

aggregation increased following woody encroachment; however, most of the C and N accumulated in free particulate organic matter (POM) fractions not protected within aggregates. Mean residence times (MRTs) of soil fractions were calculated based on changes in their $\delta^{13}C$ with time after woody encroachment. Free POM had the shortest average MRTs (30 years) and silt+clay the longest (360 years). Fine POM had MRTs of about 60 years, reflecting protection by location within aggregates. $\delta^{15}N$ values of soil fractions were positively correlated with their MRTs, suggesting that higher $\delta^{15}N$ values reflect an increased degree of humification. Increases in SOC and STN are probably being sustained by greater inputs, slower turnover of POM (some biochemical recalcitrance), and protection of organic matter in aggregates and association with silt and clay. Grassland-to-woodland conversion during the past century has been geographically extensive in grassland ecosystems worldwide, suggesting that changes in soil C and N dynamics and storage documented here could have significance for global C and N cycles.

DEDICATION

To Doss C. Barker

For believing in me

"If I have the belief that I can do it,
I will surely acquire the capacity to do it, even if I may
not have it at the beginning."
-Mahatma Gandhi

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I would like to thank Dr. Archer for the time he spent as a former committee member. I appreciate Dr. Hons and Dr. Zuberer for serving on my graduate committee. Appreciation is also expressed to Dr. Matis for much needed statistical advice in addition to being on my committee. I owe Dr. Jastrow a huge thank you for letting me spend a semester in her lab and for the time and effort she spent working with me as my DOE mentor and committee member. I specifically acknowledge the role of Dr. Boutton as my committee chair. I could not thank him enough for the countless hours he has spent helping me through this Ph.D. program. He has challenged me to make the transition from student to scholar and has offered me lots of patience, guidance, and much needed encouragement throughout the process.

Finally, I am grateful for the continued love and support of my family. I need to thank my husband, Doss Barker for his patience and encouragement. He also spent late nights keeping me company at the lab while I ran samples on the mass spec, helped me pulverize soil samples, and washed lots of glassware.

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CHAPTER I

INTRODUCTION

Numerous models have been developed to calculate the C source/sink relationship between soil and atmosphere. However, despite improvements in model accuracy, discrepancies still exist between model predictions and measurements of annual fluxes of C among the various C pools, resulting in a net imbalance of the global C budget. It has been suggested that the imbalance is attributable to terrestrial ecosystems, of which soils are a major component (Lal et al., 1995). Soil organic carbon (SOC) is the largest pool in the terrestrial C cycle and is linked to atmospheric CO_2 through inputs from plants and losses via decomposition. The annual flux of CO_2 from soil by decomposition is approximately 10 times that of emissions from fossil fuel combustion (Schlesinger, 1997). Because those flux rates are large, changes such as shifts in land cover and/or land-use practices that affect pools and fluxes of SOC have large implications for the C cycle and the earth's climate system. Increases in losses of SOC could increase atmospheric CO_2 concentrations and exacerbate global warming through positive feedback interactions, whereas sequestration of C in soils could slow the rise of atmospheric CO_2 and mitigate the effects of global warming (Lal et al., 2000).

Present assessments of the global C budget are controversial, being hampered by uncertainties in the quantitative aspects of C storage and dynamics in terrestrial

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This dissertation follows the style of the Soil Science Society of America Journal.

ecosystems. The controversy surrounding the global C budget centers primarily on whether terrestrial ecosystems are net C sources or sinks (Detwiler and Hall, 1988). Recent evidence points to a large terrestrial C sink (Houghton et al., 1999; Pacala et al., 2001). However, the geographic location as well as C accumulation rates and mechanisms controlling this terrestrial sink are largely unquantified (Houghton et al., 1999). It has been suggested that the amount of C accumulated following woody plant proliferation in non-forested ecosystems is a potentially large yet uncertain sink for atmospheric CO_2 (Houghton et al., 1999; Pacala et al., 2001).

Approximately 45 to 52% of the terrestrial surface is covered with grasslands, savannas, woodlands, shrublands, and arid lands (Daly et al., 2000). These grass-dominated ecosystems also store 30% of global SOC. Therefore, the widespread increases in woody plant abundance in many grassland and savanna ecosystems worldwide over the past century have the potential to impact the global C cycle and potentially climate. This transformation appears to be a consequence of human land-use activities, primarily fire suppression and livestock grazing (Van Auken, 2000; Archer et al., 2001). These woody plant communities are generally more productive than the grasslands they replace. Additionally, alterations in above- and belowground productivity are also accompanied by changes in plant tissue chemistry (e.g. high quality herbaceous tissue vs. poor quality lignified woody tissue) that likely alter SOC storage and dynamics (Schlesinger, 1997; Boutton et al., 1999; Archer et al., 2001).

SOC is comprised of a continuum of fractions that differ in decomposability. Pool sizes of different compartments of SOC are determined by the balance of inputs

from plants and losses via decomposition. The turnover of these soil organic matter pools is intimately linked with organic matter quality since organic matter quality plays a large role in determining turnover rates (Agren et al., 1996; Martens, 2000). It has long been recognized that there are pools or fractions of SOC that have widely different turnover rates (Jenkinson and Rayner, 1977; Paul and Juma, 1981) leading to the hypothesis that SOC can be viewed conceptually as having an active, labile pool (mean residence times (MRT's) ≈ 1-2 y), a slow pool (MRT's ≈ 25y), and a passive, recalcitrant pool (MRT's ≈ 100-1000 y) (Parton et al., 1987; Jenkinson, 1990; Schimel et al., 1994).

The chloroform-fumigation incubation method (Jenkinson and Powlson, 1976) is routinely used to determine the size of the soil microbial biomass (SMB), a component of the labile SOC pool, which is also sometimes used as a surrogate for this fast C turnover pool. Changes in the size and activity of the SMB pool has been suggested as an early indicator of the effects of changes in the quality and quantity of organic matter inputs on soil organic matter long before the effects are detectable against the large background of soil organic matter already present in the soil (Powlson et al., 1987). The ratio of SMB to total SOC (C_{mic} / C_{org}) has been used as a measure of C availability to decomposer organisms and/or the quality of the SOC (Anderson and Domsch, 1989; Sparling et al., 1992). Quantification of the microbial respiration quotient (qCO_2) reflects the ability of the SMB to convert available substrate to biomass and is an indicator of microbial efficiency (Bradley and Fyles, 1995; Wardle and Ghani, 1995).

Additional efforts to determine the composition of organic C fractions that differ in function and turnover have included both chemical and physical approaches (Christensen, 1992; Sollins et al., 1999). However, physical approaches based on density and size fractionation have recently gained popularity because they appear to be able to relate specific organic matter fractions to soil structure and aggregation. For example, greater organic matter concentrations and higher mineralization rates are reported to be associated with macroaggregate fractions and may correspond to the active, labile C pools. Conversely, organic C associated with microaggregates may be more protected physically and more recalcitrant biochemically corresponding to the slower turnover C pools (Jastrow, 1996; Jastrow et al., 1996; Jastrow and Miller, 1998; Six et al., 2000).

The N cycle is intimately linked with the C cycle due to the role of N in determining organic matter quality as indicated by C:N ratios of litter and lignin:N ratios as well as the impact of N on above- and belowground productivity (Chapin et al., 2002). Because soil N dynamics are closely coupled with soil C dynamics, physical fractionation methods may also aid in elucidating mechanisms of N accumulation in soils following land-use change. The importance of the relationships between soil organic matter and soil aggregation to C and N processes and dynamics has been underscored in the past and may provide a mechanistic explanation for soil C and N storage and dynamics following land cover or land-use changes.

In the Rio Grande Plains of southern Texas, C_4 grassland has been replaced by C_3 woodland over the past 100 years. These woodlands have greater above- and

belowground biomass and productivity than adjacent remnant grassland areas (Archer and Boutton, 1999; Boutton et al., 1999; Archer et al., 2001; Hibbard et al., 2001, 2003). In addition, several of the woody species (e.g. *Prosopis glandulosa* and *Acacia* species) are capable of symbiotic N_2–fixation and add fixed N to the system (Zitzer et al., 1996). This change in ecosystem structure has altered the balance between organic matter production and decomposition, and both C and N have accumulated in soils beneath these woodlands (Boutton et al., 1999; Archer et al., 2001; Hibbard et al., 2001, 2003). Because N is accumulating relatively more rapidly than C due to symbiotic N_2 -fixation, soil C/N ratios have also decreased over that interval (Boutton and Archer, 1998). Expanded pool sizes of soil C and N coupled with a reduction in soil C/N ratio in wooded portions of the landscape have resulted in higher rates of soil respiration and N turnover (Hibbard et al., 2001; McCulley et al., 2004).

Although the higher rates of soil respiration strongly suggest that SOC turnover is stimulated by grassland-to-woodland conversion, direct quantitative estimates of SOC turnover are lacking. In addition, there is no information regarding the quality of the organic matter that has accumulated in these soils despite the higher flux rates. Is soil C accumulating largely in active, labile pools or in inactive, recalcitrant pools? If C is accumulating primarily in active, labile forms, then perhaps SOC dynamics are accelerated where woodland replaces grassland and little net C storage is occurring. Alternatively, if C is accumulating in inactive, recalcitrant fractions, then C turnover rates may be decreased following grassland to woodland conversion. Although critical as parameters for soil organic matter models, the magnitudes and turnover times for

these active, slow, and passive soil organic matter pools are not well understood. Accurate estimates of these pool sizes and flux rates are essential for determining whether soils are net sources or sinks of atmospheric CO_2 (Falloon and Smith, 2000).

Grassland-to-woodland succession in the Rio Grande Plains of southern Texas affords a unique opportunity to use stable isotope methodology to study soil organic matter dynamics following this shift in vegetation structure. Few studies have employed both the natural abundance of ^{13}C and ^{15}N in organic matter studies. Because the current plant community is comprised of C_3 trees/shrubs ($\delta^{13}C \approx -27$ ‰) and remnant grasslands are dominated by C_4 species ($\delta^{13}C \approx -13$‰), the isotopic difference between the plant community types allows for isotopically tracing rates of SOC accumulation and turnover following grassland-to-woodland conversion, and for identifying the specific physical and chemical mechanisms by which soil organic matter is sequestered in native rangeland ecosystems that have never been cropped or cultivated. By analyzing the $\delta^{13}C$ of organic C in specific physical fractions obtained beneath woody plant stands of known age, the turnover rates of those C fractions can be estimated. The turnover rates of SOC calculated from ^{13}C dynamics of woody plant chronosequences helps provide an indication of C quality. Faster turnover indicates more labile organic matter whereas longer turnover times reflect slow or passive organic matter fractions. In particular, it will show whether SOC accumulations are the result of increases in the most recent organic matter inputs derived from woody vegetation or from retention of older organic C derived from the previous grassland, or from a mixture of both sources.

The natural abundance of ^{15}N in plants and soils may provide information on N sources and transformations in ecosystems (Michelsen et al., 1996) and is considered an integrator of soil N-cycling processes as affected by both the biotic and abiotic environment (Robinson, 2001; Dawson et al., 2002). Although multiple factors influence plant and soil $\delta^{15}N$ values such as temperature, precipitation, quantity and quality of litter inputs, soil nitrogen sources, and isotopic fractionation resulting from N-transformations (Nadelhoffer and Fry, 1988; Piccolo et al., 1994; Amundson et al., 2003), the influences of woodland development on N-cycling should be apparent in the natural abundance of ^{15}N in the plant-soil system because: (a) N-fixation adds N with a $\delta^{15}N$ value near 0‰, and (b) nearly all N-transformations lose ^{14}N-enriched N, leaving residual N relatively enriched in ^{15}N (Peoples et al., 1991).

The purpose of this study is to evaluate the biogeochemical consequences of grassland to woodland succession on the soil C and N cycles of a subtropical savanna ecosystem in the Rio Grande Plains of southern Texas. More specifically, the purpose is to determine the qualitative characteristics of SOC accumulation, and to quantify the turnover rates of these qualitatively different soil organic matter fractions following grassland-to-woodland succession. Organic matter fractionation will be employed in an effort to quantify C storage in pools known to differ in turnover rates (active, intermediate, passive pools). Specific objectives are: (1) Quantify some of the physical, chemical, and biological characteristics of SOC and evaluate how these characteristics change following grassland-to-woodland succession; (2) Quantify rates of SOC and N sequestration following grassland to woodland conversion; (3) Determine the relative

importance of C derived from woody vegetation (C_3) vs. grassland vegetation (C_4) where woodland has replaced grassland using the natural isotopic difference between C_4 grasses and C_3 woody plants; (4) Quantify turnover rates of SOC in specific soil physical fractions by compartmental analysis of $\delta^{13}C$ values; (5) Elucidate specific physical mechanisms of soil C and N sequestration in this system by determining where C and N are stored relative to soil aggregate structure; and (6) Utilize the natural abundance of ^{15}N to identify N sources, transformations, and quality of soil organic matter in soil physical fractions.

Specific information on soil organic matter dynamics is necessary in order to predict the effects of land cover changes induced by human land-use activities on C and N dynamics and to assess future land-use options associated with woody plant encroachment into grassland ecosystems in dryland regions. Knowledge of the mechanisms of organic matter incorporation into soil C pools, especially those with long turnover times, is crucial for understanding whether terrestrial ecosystem C sinks will continue to sequester C in the future. Therefore, the results of this study have the potential to validate and improve output for soil organic matter models allowing extrapolation of the results from the ecosystem-level to the broader scales needed to predict the role of terrestrial ecosystems in the global C cycle including the potential of terrestrial ecosystems for long-term C sequestration.

CHAPTER II

BELOWGROUND CARBON STORAGE AND DYNAMICS FOLLOWING WOODY PLANT ENCROACHMENT IN A SUBTROPICAL SAVANNA PARKLAND

INTRODUCTION

Woody plant encroachment has been documented in grasslands and savannas in North and South America, Australia, Africa, and Southeast Asia over the past century (Archer et al., 2001). This transformation appears to be a consequence of human land-use activities, primarily livestock grazing and fire suppression (Van Auken, 2000; Archer et al., 2001). Since 40% of the global terrestrial surface consists of grasslands, savannas, woodlands, and shrublands that store up to 30% of global soil organic C (SOC), this geographically extensive vegetation change has the potential to affect the global C budget (Scurlock and Hall, 1998; Archer et al., 2001, 2004; Goodale and Davidson, 2002; Jackson et al., 2002). Some functional consequences of woody plant expansion include alterations of above- and belowground productivity, modifications to rooting depth and distribution, and changes in the quantity and quality of litter inputs (Scholes and Hall, 1996; Connin et al., 1997; Gill and Burke, 1999; Jackson et al., 2000; Jobbágy and Jackson, 2000). Additionally, many of the woody plant species that are encroaching into grasslands are capable of symbiotic N_2-fixation, adding fixed N to N-limited ecosystems (Rundel et al., 1982; Stock et al., 1995; Zitzer et al., 1996). These alterations in vegetation, litter, and soil characteristics following woody plant encroachment into grasslands modify ecosystem C dynamics and storage and may lead

to alterations of local and regional climate systems through feedback interactions (Schlesinger et al., 1990; Ojima et al., 1999). Despite the global significance of woody plant invasion, mechanisms and processes associated with C dynamics and storage in the affected systems are poorly understood (Jackson et al., 2000, 2002; Archer et al., 2001, 2004; Hudak et al., 2003).

In the Rio Grande Plains of southern Texas, C_3 subtropical thorn woodlands dominated by highly productive N-fixing tree legumes have largely replaced C_4 grasslands over the past 100-150 years (Archer et al., 1988, 2001; Boutton et al., 1998, 1999). This vegetation change has increased rates of above- and belowground productivity and both C and N have accumulated in woodland soils (Hibbard et al., 2001; McCulley et al., 2004). These shifts in net primary productivity (NPP) are also accompanied by significant changes in plant tissue chemistry (e.g., high quality herbaceous tissue vs. poor quality lignified woody tissue) that likely alter ecosystem C storage and dynamics. Because the current plant community is comprised of C_3 trees/shrubs ($\delta^{13}C \approx -27$ ‰) and grasslands are dominated by C_4 species ($\delta^{13}C \approx -13$ ‰), this grassland-to-woodland conversion affords a unique opportunity for isotopically tracing rates of SOC accumulation and turnover in native rangeland systems that have never been cropped or cultivated.

Prior studies have demonstrated the usefulness of $\delta^{13}C$ natural abundance for estimating turnover and dynamics of SOC in soils where the photosynthetic pathway of the original vegetation has shifted (Balesdent and Mariotti, 1987; Balesdent et al., 1988; Martin et al., 1990). The $\delta^{13}C$ values of SOC reflect the plant material from which it

was derived with minimum isotopic fractionation associated with decomposition (Balesdent and Mariotti, 1987; Balesdent et al., 1988; Nadelhoffer and Fry, 1988; Martin et al., 1990). A vegetation shift from a C_4-dominated plant community to a C_3-dominated plant community or vice versa provides an *in situ* label allowing quantification of the loss rate of C derived from the previous plant community and the simultaneous accumulation of new C derived from the current plant community (Martin et al., 1990). Specific information on soil organic matter dynamics in terrestrial ecosystems is essential in order to predict the effects of land cover changes induced by human land-use practices on the C cycle of those ecosystems, to assess future land-use options associated with woody plant invasion of grasslands in dryland regions, and to assess the role of terrestrial ecosystems in the global C budget. This study seeks to enhance the understanding of the effects of land-uses (livestock production) and land cover changes (grassland-to-woodland succession) on mechanisms of soil C sequestration and turnover in rangelands.

Therefore, the objective of this study is to evaluate the impact of grassland-to-woodland conversion on the soil C cycle in the Rio Grande Plains of southern Texas. More specifically, I will: (1) Quantify rates of SOC sequestration following grassland-to-woodland conversion; (2) Determine the relative importance of residual grassland-derived C_4 carbon and the new C_3 woodland-derived carbon inputs to total SOC content in bulk soil; and (3) Utilize the natural isotopic difference between C_4 grasses and C_3 woody plants to quantify turnover rates of SOC in bulk soil.

MATERIALS AND METHODS

Study Area

Research was conducted at the Texas Agricultural Experiment Station LaCopita Research Area (27° 40'N; 98° 12'W) located 65 km west of Corpus Christi, Texas in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical, with a mean annual precipitation of 715 mm (bimodally distributed) and a mean annual temperature of 22°C. Topography consists of nearly level uplands which grade (1-3% slopes) into lower-lying drainage woodlands and playas. The elevation ranges from 75-90 m.

Originally classified as a *Prosopis-Acacia-Andropogon-Setaria* savanna (Kuchler, 1964), contemporary vegetation is subtropical thorn woodland, having experienced significant woody plant expansion in recent history. This area has been heavily grazed by domestic livestock over the past century. This land-use practice has been identified as a causal factor in succession from grassland to woodland in this region (Archer et al., 2001).

Upland soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface (B_t) horizon with non-argillic inclusions and are characterized by a two-phase vegetation pattern consisting of discrete clusters of woody vegetation embedded within a matrix of remnant C_4 grasses. Species of *Paspalum*, *Bouteloua*, *Chloris,* and *Eragrostis* dominate in the grasslands. Formation of clusters is initiated when grasslands are colonized by *Prosopis glandulosa* Torr. var. *glandulosa* (honey mesquite) which then facilitates recruitment of other woody plant species in the

understory (Archer et al., 1988). Where the argillic horizon is absent, clusters expand laterally and fuse to form larger groves of woody vegetation (Archer et al., 1995). Lower-lying portions of the landscape have clay loam soils (Pachic Argiustolls) and are characterized by continuous closed-canopy drainage woodlands. The vegetation composition of these drainage woodlands is similar to that in upland clusters and groves. *Prosopis glandulosa* is the dominant species in all wooded landscape elements with *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory.

Chronosequence Approach

A chronosequence approach was used to quantify C pool sizes and their rates of change in litter, roots, and soils following woody plant encroachment into areas that were previously grassland. Ten sites were sampled within remnant grasslands, clusters, and groves; 11 sites were sampled within drainage woodlands. All sites were located within an area of approximately 2 km^2. Remnant grasslands were sampled to characterize C pool sizes at time zero (i.e. prior to woody encroachment), whereas clusters, groves, and drainage woodlands of known age were sampled to evaluate changes in C pools at different points in time following woody invasion.

Ages of clusters, groves, and drainage woodlands were based on the fact that the formation of these wooded landscape elements is initiated only after the establishment of *Prosopis glandulosa* in grassland (Archer et al., 1988). Thus, the age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand. The ages of *P. glandulosa* trees were determined by measuring their basal diameters, and then

substituting those values into regression equations to predict tree ages using equations specific to each landscape element (Stoker, 1997). Woody plant stands sampled in each of the landscape elements were selected to encompass the full range of *P. glandulosa* basal diameters, corresponding to tree ages ranging from approximately 10-130 years.

Collection of Soil, Root, and Litter Samples

Surface litter was collected from a 0.25-m^2 quadrat at each site. In wooded landscape elements, the quadrat was located within 0.5 m of the bole of the largest *P. glandulosa* tree. Litter samples were washed with water over a 2-mm sieve to remove adhering soil particles, dried at 60^{o}C to constant weight, weighed, pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI), and saved for elemental and isotopic analyses.

Four soil cores (5-cm diameter x 30-cm depth) were taken within 0.5 m of the bole of the largest *P. glandulosa* at each wooded site, one in each cardinal direction from the bole. Sampling was identical in each remnant grassland site, but instead centered around the base of a large perennial C_4 grass plant. Soil surface litter was removed gently to expose mineral soil prior to taking each soil core. All soil cores were sectioned into 0-15 and 15-30 cm increments and stored at 4^{o}C. In the lab, each soil sample was mixed thoroughly, and a subsample was dried at 105^{o}C to determine bulk density. Then, the 4 cores from each site were pooled by depth increment and mixed.

Prior to further treatment, an aliquot of each soil sample was set aside for determination of root biomass. Another aliquot was passed through a 2-mm sieve to remove large organic fragments and used for physical, chemical, and isotopic analyses.

Soil pH was determined on a 1:2 (soil:water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipet method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was dried at 60°C, pulverized in a centrifugal mill, and used for elemental and isotopic analyses. Root biomass was quantified on well-mixed, aliquots (100 g) from each pooled soil sample using a hydropneumatic elutriation system (Smucker et al., 1982) (Gillison's Variety Fabrication, Inc., Benzonia, MI) equipped with a 410-μm screen. Roots were then dried at 60°C, weighed, pulverized with a mortar and pestle, and saved for elemental and isotopic analyses.

Elemental and Isotopic Analyses

Litter, root, and soil samples were analyzed for C concentrations and for $\delta^{13}C$ values using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (ThermoFinnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Carbon isotope ratios are presented in δ-notation:

$$\delta^{13}C = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^3 \quad (1)$$

where R_{SAMPLE} is the $^{13}C/^{12}C$ ratio of the sample, and R_{STD} is the $^{13}C/^{12}C$ ratio of the V-PDB standard (Coplen, 1996). Precision of duplicate measurements was 0.1‰. None of the samples contained $CaCO_3$ or other forms of inorganic carbon.

The relative proportion of SOC derived from the original C_4 grassland vegetation (F_C) vs. the more recent C_3 woodland vegetation was estimated by mass balance:

$$F_C = (\delta_T - \delta_{WL}) / (\delta_{SOMG} - \delta_{WL}) \quad (2)$$

Where δ_T is the $\delta^{13}C$ value of SOC at time T, δ_{WL} is the average $\delta^{13}C$ value of organic matter inputs (litter and roots) in woodlands (-26 ‰), and δ_{SOMG} is the average $\delta^{13}C$

value of SOC in remnant grasslands (-17.6 ‰ at 0-15 cm, and –14.9 ‰ at 15-30 cm).

Statistical Analyses

ANOVA was used to test for differences in soil pH, bulk density and particle size distribution with respect to landscape element and soil depth. Regression analyses were performed using Sigma Plot 4.0 (SPSS Inc., 1997) to determine the significance of relationships between C storage in surface litter biomass, root biomass, and soil with woody plant stand age. ANOVA (NCSS, 1995) was used to analyze differences in litter, root, and SOC stocks (g C m^{-2}) and $\delta^{13}C$ values attributable to the effects of landscape element and soil depth. Fisher's LSD method was used to distinguish differences among landscape elements within each soil depth following ANOVA. Exponential relationships between soil $\delta^{13}C$ and F_C and woody plant stand age were established by nonlinear curve fitting. Fractional rate constants (*k*-values) for organic C decay were obtained by fitting exponential equations of the form $y = e^{-kt}$ to the F_C data, and mean residence times (MRTs) were computed as the inverse ($1/k$) of the fractional rate constants. Relationships between F_C and stand age were forced through $F_C = 1$ at time zero. All statistical results reported are significant at $p < 0.05$.

RESULTS

Soil Characterization

Soil pH was approximately 6.5 in the upper 15 cm of the profile in remnant grasslands and was not altered following woody plant encroachment into grasslands (Fig. 1). Bulk density of the 0-15 cm depth interval decreased significantly from 1.18 in

grasslands to 1.02 in wooded landscape elements (clusters, groves, and drainage woodlands); at 15-30 cm, bulk densities ranged from 1.20-1.25 g cm^{-3}, but differed only between grassland and drainage woodland. Particle size distribution in upland soils (grasslands, clusters, and groves) at 0-15 cm was approximately 80% sand, 10% silt, and 10% clay. In contrast, the particle size distribution of lower-lying drainage woodland soils was 60% sand, 20% silt, and 20% clay (Fig. 1). Patterns were similar at 15-30 cm, although all soils at this depth contained slightly more silt and clay relative to 0-15 cm.

Carbon Storage in Litter, Roots, and Soils

Surface litter C stocks ranged from 100-175 g C m^{-2} and were not significantly different among grassland, cluster, and drainage woodland landscape elements (Fig. 2). In contrast, the litter C stock in groves (310 g C m^{-2}) was significantly greater than that of litter C stocks in all of the other landscape elements. Carbon stocks in roots (0-30 cm) were significantly greater in all wooded landscape elements (800-1500 g C m^{-2}) compared with remnant grasslands (<400 g C m^{-2}). Total SOC in the upper 15 cm of the soil profile increased from 1000 g C m^{-2} in remnant grasslands to 3000 g C m^{-2} in drainage woodlands. Total SOC at 15-30 cm was not significantly different among the upland landscape elements, but drainage woodlands contained twice as much C as found in the upland portions of the landscape (Fig. 2).

Rates of Carbon Sequestration in the Soil System

Carbon stocks in surface litter increased linearly over time following woody plant encroachment (i.e. with increasing woody plant stand age) from approximately 100 g C m^{-2} in woody plant stands < 30 years old to nearly 400 g C m^{-2} in stands > 60 years

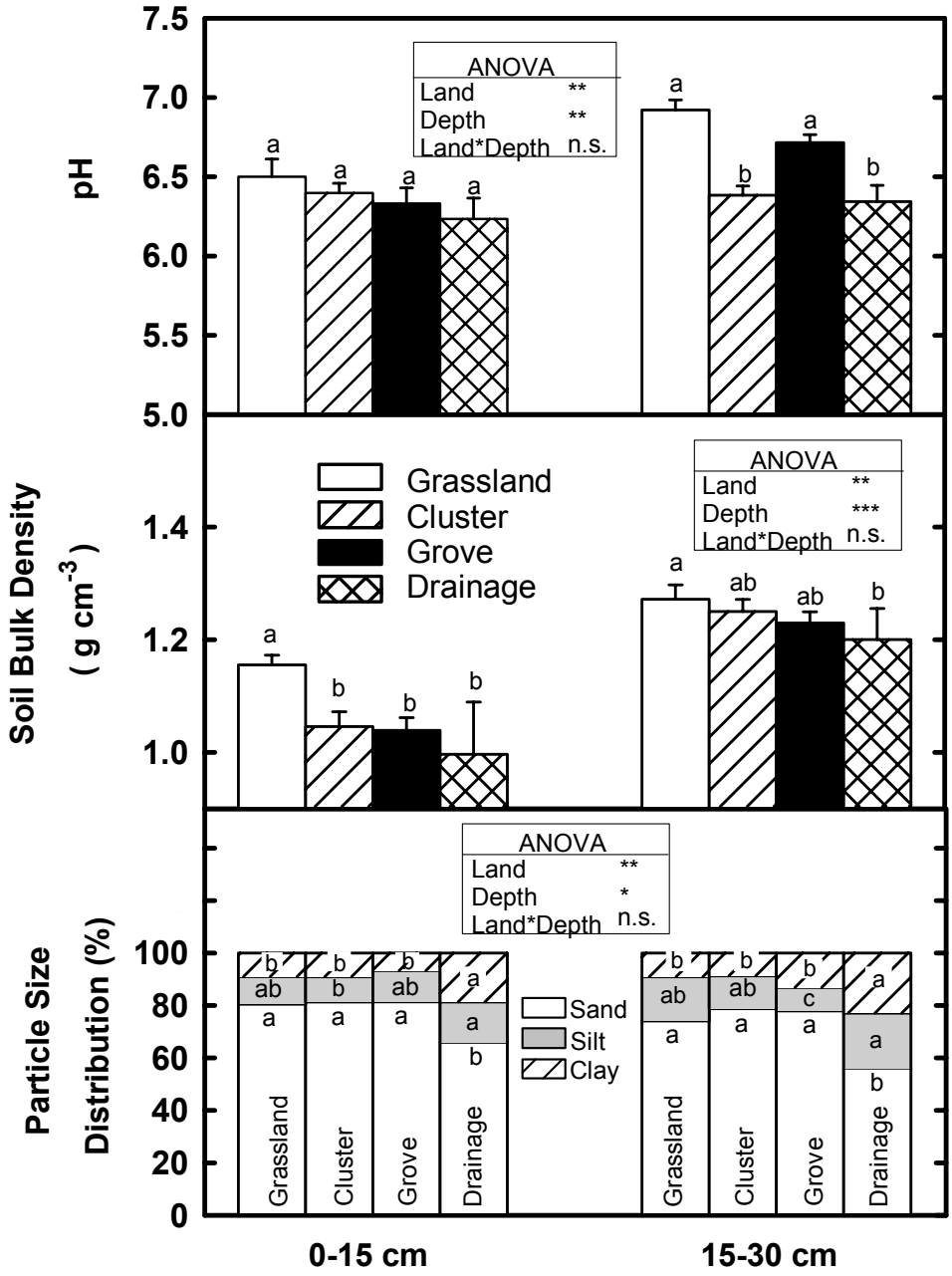

Fig. 1. Texture, bulk density, and pH of soil in grassland, cluster, and drainage woodland landscape elements at LaCopita Research Area. Error bars represent standard errors of the mean. Asterisks denote level of significance from ANOVA ($p<0.05$*, $p<0.01$**, $p<0.001$***, n.s.= not significant at $p<0.05$). Different letters above/within bars indicate differences between means within a depth from Fisher's LSD.

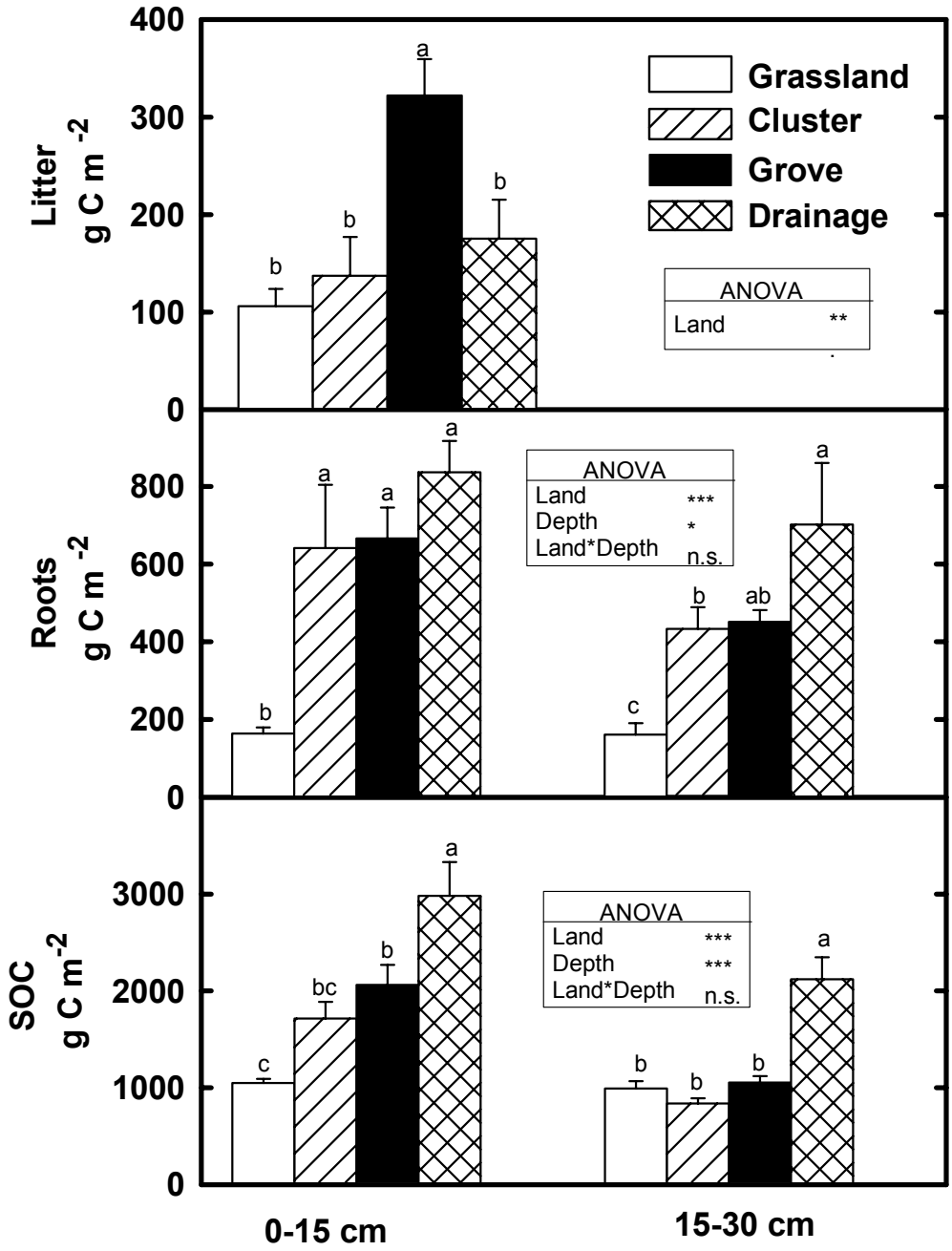

Fig. 2. Litter, roots, and SOC of soils in different landscape elements at LaCopita Research Area by soil depth (0-15 cm and 15-30 cm). Error bars are standard errors of the mean. Asterisks denote level of significance from ANOVA ($p<0.05$*, $p<0.01$**, $p<0.001$***, n.s. = not significant at $p<0.05$). Different letters above bars indicate significant differences among means within a depth from Fisher's LSD.

old (Fig. 3). Similarly, C stocks in root biomass (0-15 cm) increased linearly from 200 g C m^{-2} in remnant grassland to > 1000 g C m^{-2} in some of the older (i.e. 60-130 years) woody plant stands. These dramatic increases in litter and root C are associated with equally dramatic increases in SOC stocks at 0-15 cm from 1000 g C m^{-2} in grasslands to > 4000 g C m^{-2} in drainage woodlands. Over the past century, SOC accumulation rates (upper 30 cm of profile) have ranged from 11.5 g C m^{-2} yr^{-1} in upland clusters to 43.2 g C m^{-2} yr^{-1} in low-lying drainage woodlands, and most of this soil C accumulation (70-90%) was stored in the upper 15 cm of the profile (Fig. 3; Table 1). For the soil system (litter + roots + soil), rates of C sequestration ranged from 21 to 62 g C m^{-2} yr^{-1} in wooded landscape elements. Less than 10% of C-accumulation rates in the whole-soil system could be attributed to C-storage in litter whereas 25-45% of C-accumulation rates in the whole-soil system could be accounted for by C-accumulation in roots. Most of the accumulation in the soil system (55-70%) was due to C-storage in soil (Table 1).

Changes in the Natural Abundance of ^{13}C in Litter, Roots, and SOC

The average $\delta^{13}C$ value of grassland litter was –17 ‰, which was significantly higher than the average $\delta^{13}C$ value of −26 ‰ for woodland litter (Fig. 4). Litter $\delta^{13}C$ values in wooded landscape elements had strongly C_3-dominated signatures (−25 to –26 ‰) and did not differ significantly among cluster, grove, and drainage woodland. Root $\delta^{13}C$ values at both soil depths averaged –18 ‰ in grasslands and –26 ‰ in woodlands. Both litter and root $\delta^{13}C$ values indicated a predominance of C_3 plant inputs in the wooded landscape elements, and C_4 plant inputs in the grasslands. Mean $\delta^{13}C$

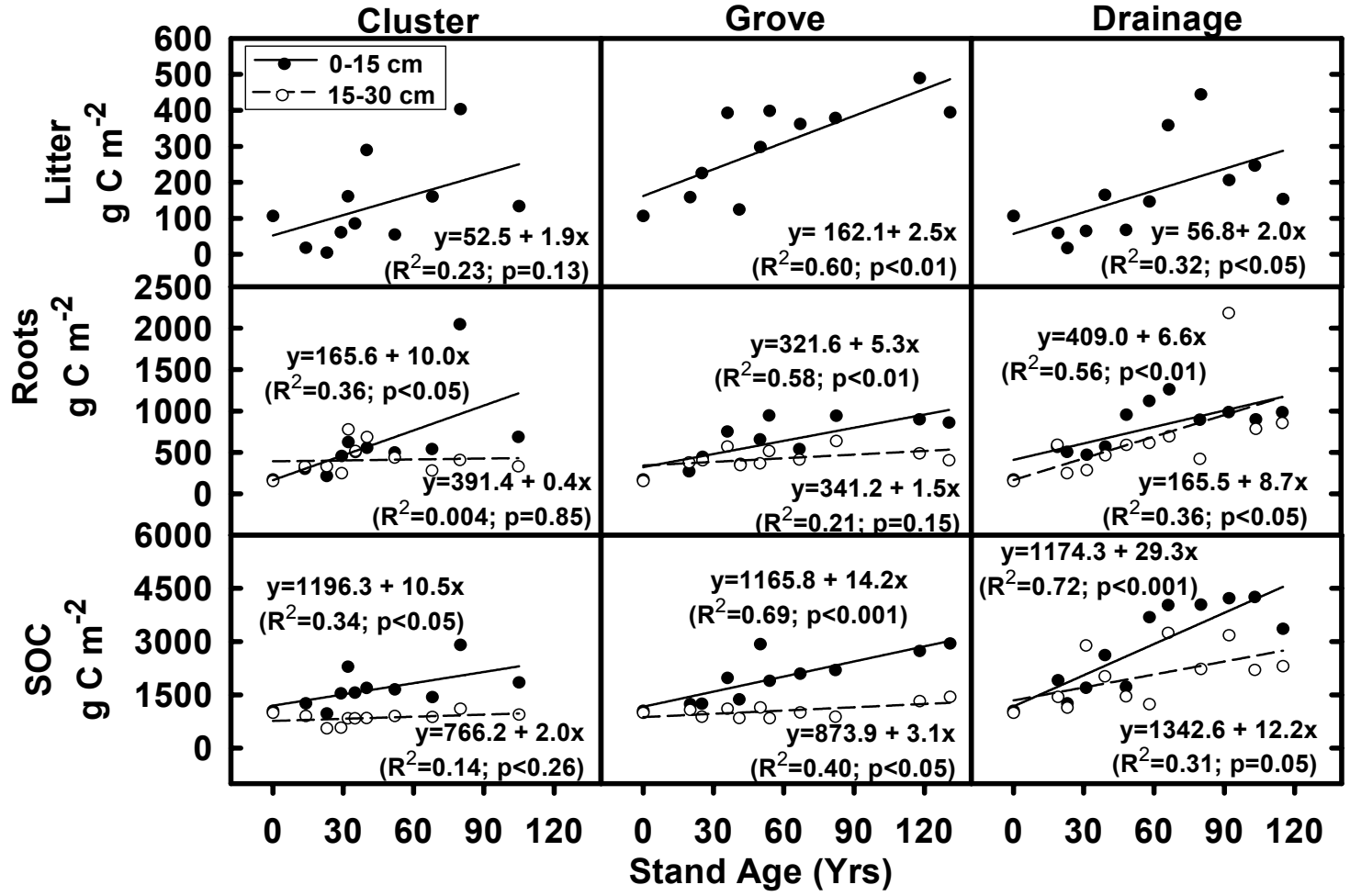

Fig. 3. Changes in litter, roots, and SOC of soils (0-15 cm and 15-30 cm depths) with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at time zero and represent baseline starting values.

Table 1. Accumulation rates of C in litter, roots, soil, and the total soil system (litter + roots + soil). Rates of accumulation in litter, roots, and soil are derived from the slopes of the regression lines in Fig. 3. Rates from 0-30 cm for the total soil system were obtained by performing separate regressions on summed data. Asterisks denote significance of the linear regression at $p<0.05$.

	Accumulation Rates (g C m^{-2} yr^{-1})							
	Litter	Roots			Soil			Total
		0-15	15-30	0-30	0-15	15-30	0-30	
Cluster	1.9*	10.0*	0.4	9.2*	10.5*	2.0	11.5*	20.5*
Grove	2.5*	5.3*	1.5	9.6*	14.2*	3.1*	17.2*	29.4*
Drainage	2.0*	6.6*	8.7*	16.1*	29.3*	12.2*	43.2*	61.9*

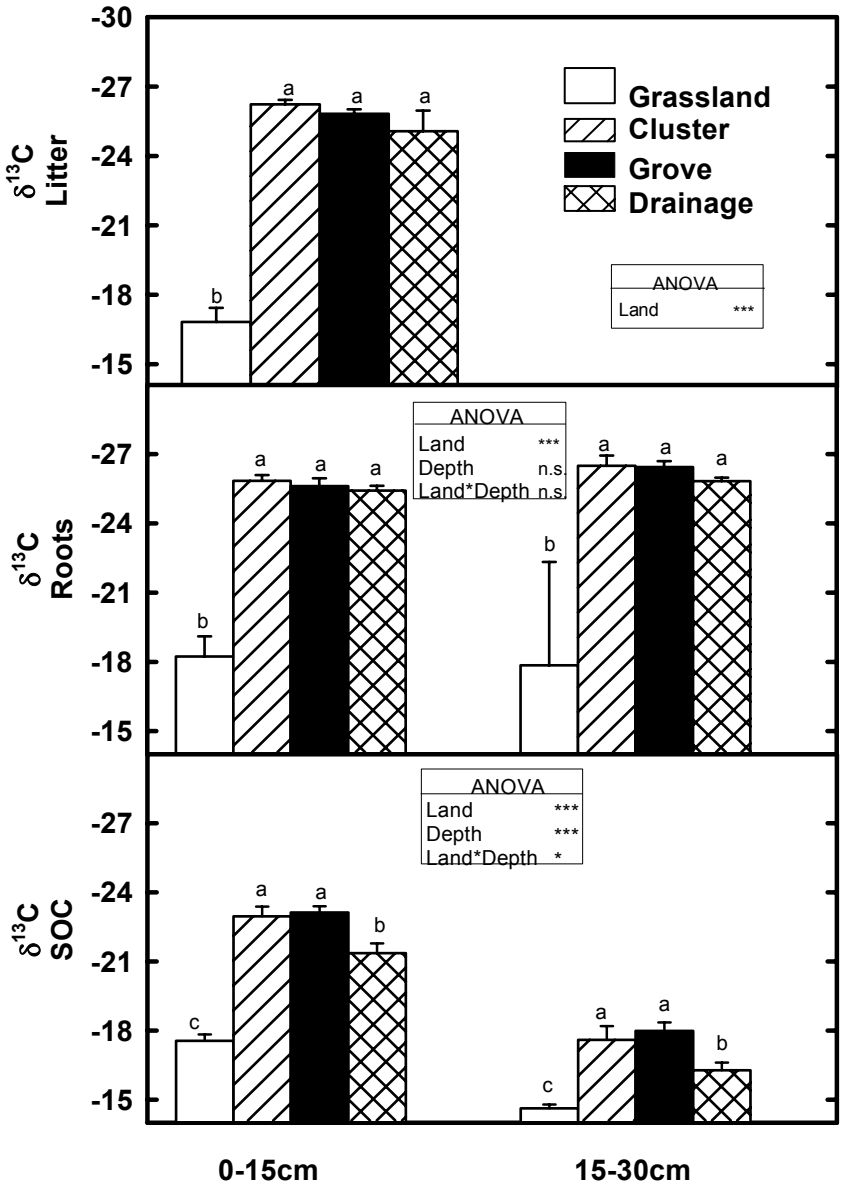

Fig. 4. $\delta^{13}C$ values of litter, roots, and SOC in different landscape elements at LaCopita Research Area by soil depth (0-15 cm and 15-30 cm). Error bars are standard errors of the mean. Asterisks denote level of significance from ANOVA ($p<0.05$*, $p<0.01$**, $p<0.001$***, n.s.=non significant at $p<0.05$). Different letters above bars indicate significant differences among means within a depth from Fisher's LSD.

value of woodland SOC at 0-15 cm depth was -23 ‰, which was significantly lower than the average remnant grassland value of -18 ‰. The average $\delta^{13}C$ value of grassland SOC at 15-30 cm was -15 ‰, which was significantly higher than $\delta^{13}C$ values for wooded areas at the same soil depth (-17 ‰) (Fig. 4).

$\delta^{13}C$ values of SOC in the upper 15 cm of the soil profile decreased exponentially over time following woody plant encroachment (i.e. with increasing woody plant stand age) from approximately –18 ‰ in remnant grasslands (time 0) to –24 ‰ in upland clusters and groves (Fig. 5). In drainage woodlands, $\delta^{13}C$ values of SOC (0-15 cm) decreased over time from –18 ‰ to –22 ‰. These isotopic changes largely occurred within 60 years of woody plant encroachment into grasslands, and are a consequence of the simultaneous loss of grassland-derived C and the gain of woody plant-derived C following grassland-to-woodland conversion. In the 15-30 cm depth increment, $\delta^{13}C$ values of SOC decreased exponentially from –15 ‰ in remnant grasslands to approximately –19 ‰ in upland clusters and groves. In contrast, $\delta^{13}C$ values at 15-30 cm in drainage woodlands changed little with time following woody encroachment.

An isotopic mass balance model was utilized to compute the relative proportions of SOC derived from grassland vs. woodland (Eq. 2; Fig. 5). According to this model, the fraction of SOC derived from grassland (F_C) in the 0-15 cm increment decreased from 1.0 at time 0 to approximately 0.2 within 100 years after cluster and grove establishment; in contrast, the fraction of C derived from grassland decreased from 1.0 to approximately 0.4 in drainage woodlands over the same time period (Fig. 5). In the 15-

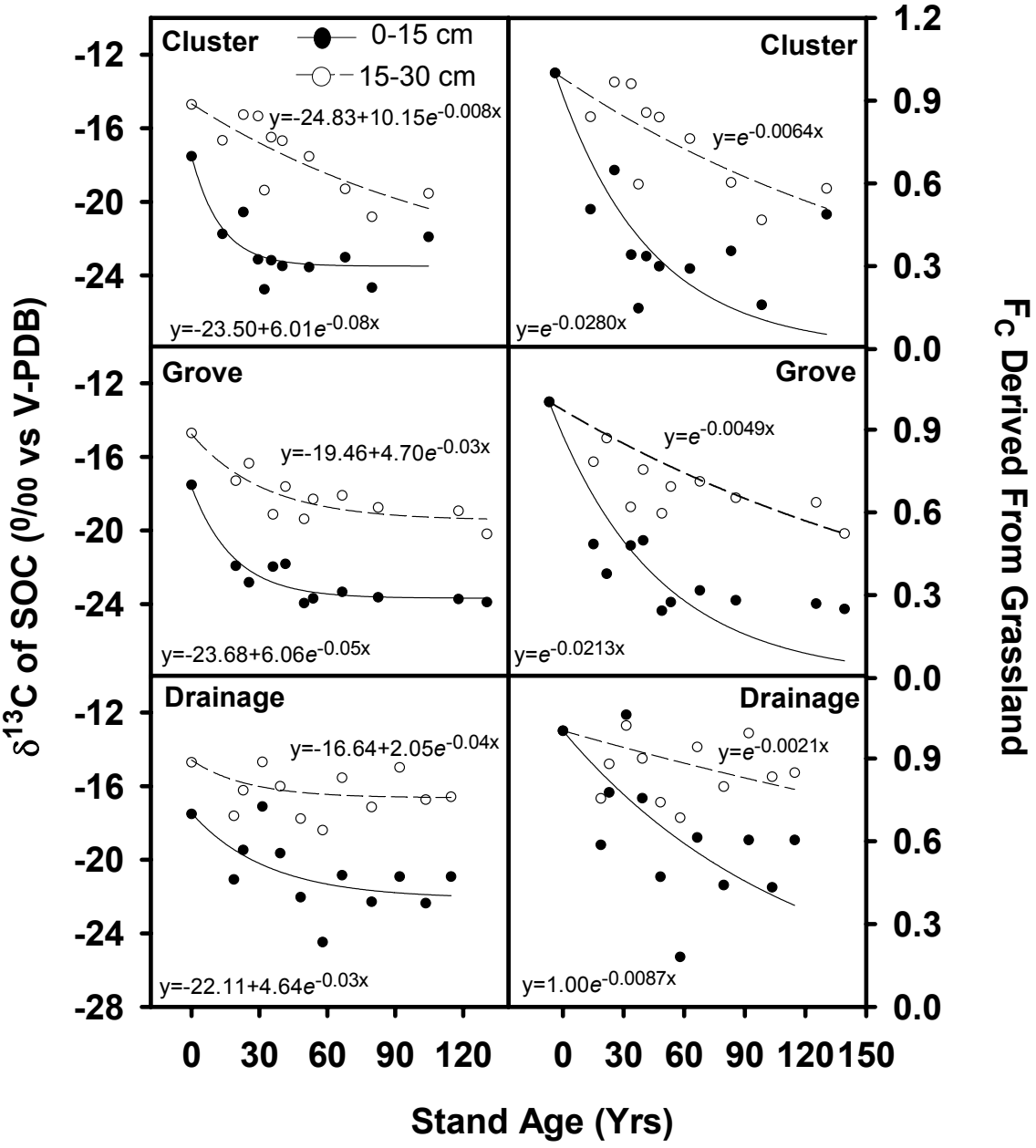

Fig. 5. $\delta^{13}C$ values of SOC (left axis) and fraction of SOC (F_C) derived from grassland (right axis) by soil depth (0-15 cm and 15-30 cm) and woody plant stand age. Time zero in each frame is based on values for remnant grasslands.

30 cm depth increment, the fraction of soil C derived from grassland decreased to 0.5 in clusters and groves and to 0.8 in drainage woodlands.

Fractional rate constants (*k*-values) for organic C decay were obtained by fitting exponential equations of the form $y = e^{-kt}$ to the F_C data in Fig. 5, and mean residence times (MRTs) were computed as the inverse ($1/k$) of the fractional rate constants (Table 2). MRTs of SOC at 0-15 cm in upland clusters (36 years) and groves (47 years) were lower than those for drainage woodlands (115 years). MRTs were substantially greater at 15-30 cm in the profile, ranging from 156 years in clusters to 476 years in drainage woodlands.

Tracing Sources of SOC Following Grassland-to-Woodland Conversion

Changes in the density (g C m^{-2}) of whole-soil organic C resulting from the decay of C inherited from the original C_4-dominated grassland and the simultaneous accumulation of C derived from C_3 woodland over the past century (Fig. 6) were revealed by multiplying age-specific fractions of C derived from grassland (F_C) (Fig. 5) by the age-specific soil C pool sizes (Fig. 3). The rate of accretion of SOC derived from C_3 woody plants was greater than the rate of loss of organic C derived from the original grassland, so that overall SOC increased by 100-200% in upland clusters and groves, and by >300% in drainage woodlands (Fig. 6).

DISCUSSION

Linear increases in C storage in litter, roots, and soil demonstrate that woody plant invasion of grassland has resulted in significant C sequestration over the past

Table 2. Comparison of *k*-values and MRTs of SOC in cluster, grove, and drainage landscape elements at LaCopita Research Area. MRTs are computed as the inverse of the fractional rate constants (*k*) in the exponential equations in Fig. 5. derived from ^{13} C isotope data. Numbers in parentheses are the standard errors of the estimates. MRTs obtained from ^{14}C radiocarbon dating are from Boutton et al. (1998).

	***k*-values**		**C-13 Mean Residence Times (yrs)**		**C-14 Mean Residence Times (yrs)**	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Cluster	0.0280 (0.0053)	0.0064 (0.0010)	36	156		
Grove	0.0213 (0.0029)	0.0049 (0.0005)	47	204	52 (17)	282 (77)
Drainage	0.0087 (0.0018)	0.0021 (0.0006)	115	476	54 (12)	516 (188)

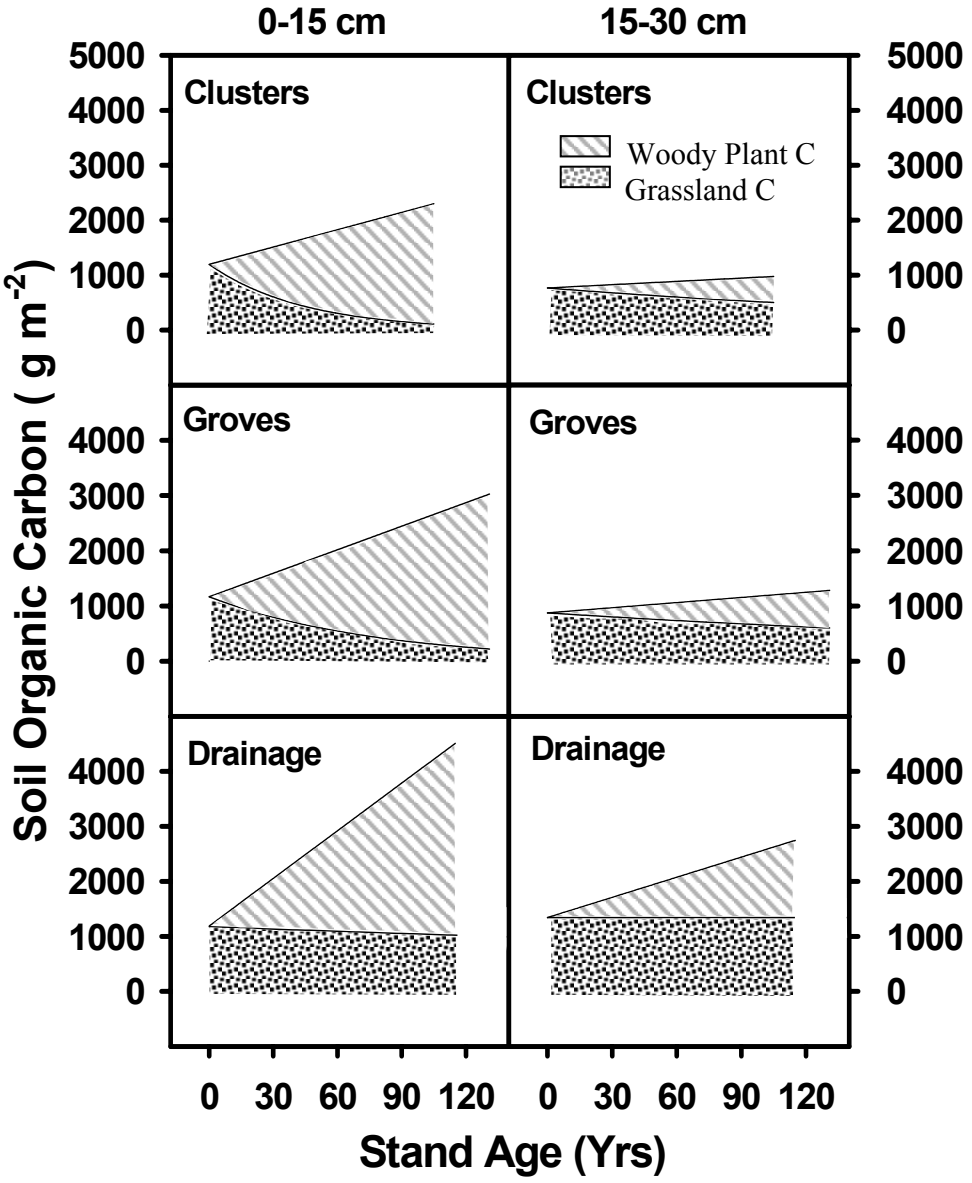

Fig. 6. Changes in the mass of total SOC resulting from the decay of SOC inherited from the original C_4 grassland and the simultaneous accumulation of SOC derived from C_3 woodland development over the past century. Values of total SOC were obtained by direct measurement. The mass of SOC from grassland was calculated as the product of total SOC and F_C from Fig. 5. The mass of SOC from C_3 woodland was calculated as the difference between total SOC and the mass of SOC from grassland.

century (Fig. 3). Most of the C accumulation in roots and SOC was in the upper 15 cm of the soil profile. Values for grassland SOC were 1000 g C m^{-2}, while values for the oldest wooded areas ranged from 3000 to 4500 g C m^{-2}, representing an increase of 200-350% (Fig. 3). This dramatic increase in SOC is likely a consequence of higher rates of net primary productivity (NPP) in the wooded landscape elements compared to remnant grasslands. Aboveground NPP has increased from 1.9-3.4 Mg ha^{-1} yr^{-1} in remnant grasslands to 5.1-6.0 Mg ha^{-1} yr^{-1} in areas now dominated by woody vegetation (Archer et al., 2001). Although belowground productivity has not been quantified, several studies have shown significantly greater root biomass and seasonal fluctuations in root biomass in wooded areas than in remnant grasslands, providing strong circumstantial evidence for higher belowground productivity in wooded portions of the landscape (Boutton et al., 1999; Hibbard et al., 2001).

SOC accumulation in wooded areas may also occur due to the poorer quality of organic matter inputs in these landscape elements. This is a plausible mechanism as the chemical composition of shrub leaf litter is thought to be more resistant to decay due to increased presence of lignin, tannins, and other 2^{o} compounds that are not present in herbaceous grassland litter (Horner et al., 1988; Enríquez et al., 1993; Gillon et al., 1994; Sharma et al., 1995; Hobbie, 1996; Köchy and Wilson, 1997; Kraus et al., 2003). Prior results from long-term incubation studies (Boutton et al., 2002) and from field studies of soil respiration (McCulley et al., 2004) showed that turnover of SOC was slower in woodlands than in remnant grasslands, suggesting that organic matter quality may have decreased following grassland-to-woodland conversion.

In other studies, responses of SOC to woody encroachment into grasslands have ranged from no change to significant increases. Most studies across comparable arid/semi-arid ecosystems have shown that woody plant invasion of grasslands results in increased C-storage in soil beneath woody plant canopies (Virginia and Jarrell, 1983; Mordelet et al., 1993; Stock et al., 1995; San Jose et al., 1998; Geesing et al., 2000; Burrows et al., 2002; Reyes-Reyes et al., 2002). In contrast, a few recent studies have shown no changes in SOC (McCarron et al., 2003; Smith and Johnson, 2003) or actual decreases in SOC with woody plant encroachment (Jackson et al., 2002).

It is not yet clear why responses to woody encroachment are variable and range from net losses to net gains in soil C. Documented variable effects of woody plant impacts on SOC may be due to several factors such as species dependent changes in microclimate, litter production, litter quality, and rooting depth and biomass (Bush and Van Auken, 1986; Lugo and Brown, 1993; Pugnaire et al., 1996; Jackson et al., 2000). However, in many of the studies in which significant increases in SOC were observed, encroaching woody plant species were often N-fixing tree legumes (Virginia and Jarrell, 1983; Bush and Van Auken, 1986; Stock et al., 1995; Pugnaire et al., 1996). Greater inputs of fixed-N to N-limited ecosystems reduce N-limitation of plants and microbes and results in greater plant productivity (Wardle, 1992). Because N is limiting in most ecosystems, the presence of N-fixing species may be an important driving factor in the observed increases in SOC in many aridland ecosystems. However, the discrepancies among studies suggests that current understanding of the effects woody plant species on grassland biogeochemistry is still limited, highlighting the need for additional

ecosystem-level studies of SOC storage and dynamics following grassland-to-woodland conversion.

Although many studies have addressed changes in pool sizes of SOC following grassland-to-woodland conversion, few of these studies have determined rates of C-accumulation in soil or in the total soil system (litter + roots +soil). C-accumulation in the total soil system ranged from 21-62 g C m^{-2} yr^{-1} (Table 1). These rates reflect the impact of the higher quantity of organic matter inputs to soil as a result of increased above- and belowground productivity of woodlands relative to remnant grasslands. SOC accumulation rates in this study ranged from 12-43 g C m^{-2} yr^{-1} and accounted for 55-70% of C accumulation in the belowground system. C-accumulation in litter accounted for less than 10% of C-accumulation in the total soil system whereas roots accounted for 25-45% of the observed C-accumulation rates in the total soil system (Table 1). This is consistent with the observation that root productivity is greater than litterfall in wooded areas at this site (Hibbard et al., 2001).

Rates of soil C accumulation were greatest in drainage woodlands and lower in clusters and groves. This higher accumulation rate of SOC in drainage woodlands may be related to the higher rates of NPP due to the more favorable soil moisture regimes in this lower-lying portion of the landscape. Additionally, the finer-textured soils (higher silt and clay content) in drainage woodlands (Fig. 1) may better stabilize organic matter, allowing greater protection from decomposition by soil microbes through the formation of macro- and microaggregates (Ladd et al., 1993). Conversely, coarse-textured soils of upland clusters and groves have less potential to form stable aggregates and protect

organic matter from decomposition, resulting in slower SOC accumulation rates in the upland woodlands.

Rates of C accumulation documented in this study are comparable to those obtained from prior field and modeling studies at this same site. Model output from CENTURY simulations estimated a relatively narrow range of accumulation rates (11-19 g C m^{-2} yr^{-1}) for SOC in wooded landscape elements (0-20 cm) at this study site (Hibbard et al., 2003). However, results based on field measurements to a depth of 20 cm showed rates ranging from 12-47 g C m^{-2} yr^{-1} (Archer et al., 2001, 2004) which are virtually identical to the accumulation rates found in this study (12-43 g C m^{-2} yr^{-1}). These rates are also similar to SOC accumulation rates found in other ecosystems experiencing woody plant encroachment or afforestation. An average accumulation rate of 30 g C m^{-2} yr^{-1} was determined for soils (0-30 cm) of a savanna ecosystem in Africa experiencing grassland-to-woodland conversion (Scholes and van der Merwe, 1996). Carbon accumulation rates documented in the present study are also within the range of observed rates for abandoned agricultural land in both temperate and tropical regions. A review by Silver et al. (2000) showed SOC accumulation rates of approximately 41 g C m^{-2} yr^{-1} following 100 years of forest re-establishment on abandoned agricultural and pasture lands in the tropics. Schlesinger (1997) reported rates of SOC accumulation of 21-55 g C m^{-2} yr^{-1} in abandoned agricultural soils and other disturbed sites allowed to return to native vegetation in temperate regions.

Although SOC is accumulating in soils following woody plant invasion of grassland, the mechanisms of this accumulation are poorly understood. The $\delta^{13}C$ natural

abundance method is useful for estimating turnover and dynamics of SOC in soils and in elucidating mechanisms of SOC accumulation where the photosynthetic pathway of the original vegetation has shifted (Balesdent and Mariotti, 1987; Balesdent et al., 1988; Martin et al., 1990). Prior to woody invasion, grassland soils had $\delta^{13}C$ values ranging from -17 to -18 ‰, typical of C derived from C_4 grassland (Boutton et al., 1999). Following woody plant invasion of grassland, $\delta^{13}C$ of SOC declined in negative exponential fashion, reflecting the simultaneous loss of C derived from the original C_4 grassland and the accumulation of C derived from the current C_3 woody vegetation. To determine the relative proportions of SOC derived from the original C_4 grassland vegetation (F_C) vs. the more recent C_3 woodland vegetation, mass balance calculations were used. After 130 years of woodland development, 10-30% of C_4-derived C remained in the soil. This fraction of grassland-derived SOC appears to be relatively resistant to decay as little change is detected in the magnitude of this pool in woody plant stands older than about 60 years (Fig. 5). At 15-30 cm, 60-80% of C derived from grassland remained present in the soil even after 130 years of woodland development, suggesting that this fraction is resistant to decomposition and is an important pool for long-term C storage. These results are comparable to the findings of Martin et al. (1990) for a tropical savanna ecosystem in Ivory Coast where C_3 woody plants have invaded grasslands dominated by C_4 grasses. Martin et al. (1990) found that 30-50% of the original grassland-derived SOC in the upper 10 cm of the profile remained following 16 years of woody plant development, whereas 60-70% of the original grassland-derived SOC remained at 10-25 cm in the profile.

The rate of change of the $\delta^{13}C$ values of SOC following woody invasion is a direct function of the SOC turnover rate. MRTs were computed as the inverse ($1/k$) of the fractional rate constants for organic C decay obtained by fitting exponential equations to the F_C data in Fig. 5. MRTs for SOC ranged from 36-115 years for SOC at 0-15 cm in woodlands. At 15-30 cm, MRTs were much longer, ranging from 156-476 years. The longer MRTs associated with depth may be related in part to the higher silt and clay contents deeper in the soil profile (Fig. 1). Organic C associated with silts and clays generally have slower turnover rates than those associated with sand-size fractions (Christensen, 1992). Carbon that is adsorbed onto mineral surfaces is relatively inaccessible to microbial decay and may thus persist in the soil resulting in increased MRTs (Baldock and Skjemstad, 2000). A previous study at LaCopita Research Area indicated that a large proportion of old C_4-derived C has persisted in the clay fraction of woodland soils likely due to protection of the organic matter from microbial decomposition by association with the soil mineral fraction (Boutton et al., 1998).

MRTs for SOC in woodlands in this study were comparable to estimates obtained from ^{14}C dating of woodland derived SOC in an earlier study (Boutton et al., 1998). The natural abundance of ^{13}C appears to be a feasible and relatively accurate means of estimating residence times of SOC in whole soil pools following woody plant invasion of grassland. This is contrary to the results obtained by Paul et al. (2003), which showed that MRTs obtained by natural ^{13}C appeared to grossly underestimate SOC turnover rates derived from radiocarbon dating. MRTs could not be determined for SOC in grasslands using the natural abundance of ^{13}C method due to the lack of a tracer.

Overall, evidence from ^{13}C dynamics show that the rate of accretion of C derived from C_3 woody plants is greater than the loss of organic C derived from the original grassland so that SOC in woodlands has increased significantly relative to remnant grassland (Fig. 6). Thus, it appears that soils have been actively sequestering C over the past century following woody plant invasion of grassland. Although the findings of this study along with many others show increasing SOC with woody plant encroachment into grasslands, the consequences of woody encroachment on soil C pools and fluxes in affected ecosystems remain controversial. Current estimates suggest that woody plant encroachment into grasslands and savannas may result in the sequestration of 0.10-0.13 Pg C yr^{-1} in the US which represents 20-40% of the current US carbon sink strength (Houghton et al., 2000; Tilman et al., 2000; Pacala et al., 2001). If these estimates are correct, then woody encroachment is certainly playing a significant role in the global C cycle. However, neither the geographic extent nor the ecosystem level impacts on C storage are well constrained at this time. There remains a need for local and regional assessments of SOC pools and fluxes in order to extrapolate results from the ecosystem-level to the broader scales needed to predict the role of terrestrial ecosystems in the global C budget including the potential of terrestrial ecosystems for long-term C storage.

CONCLUSIONS

Although woody plant invasion of grasslands has been geographically extensive over the past century, little is known regarding the biogeochemical consequences of this land cover change. This study indicates that woodlands have replaced grasslands over the past century in the Rio Grande Plains of southern Texas. Following this grassland-

to-woodland transition, C storage in litter, roots, and soil has increased significantly concomitant with increases in above- and belowground productivity. Accumulation rates of SOC ranged from 12-43 g C m^{-2} yr^{-1} consistent with prior results obtained from field estimates and modeling at this same site, and comparable to estimates from other ecosystems experiencing woody plant encroachment or from reforestation of agricultural lands. C storage and dynamics in surface soils are driven primarily by C_3 woody inputs. Additionally, it appears that a large portion of SOC in woodlands, 10-20% in surface soils and 60-80% at deeper depths, is comprised of older C derived from the previous grassland community that is relatively resistant to decay as little change was detected in this pool following 60-130 years of woodland development. Hence, accumulations of SOC are due to both increased inputs of newer woodland-derived C and the retention of older, stabilized grassland-derived C. The significant increases in SOC documented in this study suggest that these areas in southern Texas undergoing grassland-to-woodland conversion have been acting as a net sink for atmospheric CO_2 over the past century. Grassland-to-woodland conversion over the past century has been geographically widespread in the world's drylands suggesting that changes in SOC storage and dynamics documented here could have significance for regional and global C cycles and potentially climate.

CHAPTER III

CHANGES IN SOIL NITROGEN STORAGE AND $\delta^{15}N$ WITH WOODY PLANT ENCROACHMENT IN A SUBTROPICAL SAVANNA PARKLAND LANDSCAPE

INTRODUCTION

Woody plant encroachment into grass-dominated systems has been among the most important global land cover changes over the past 100-200 years (Archer et al., 2001). This widespread shift in land cover is generally attributed to human land use activities, particularly livestock grazing and fire suppression (Archer et al., 2001). In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by N-fixing tree legumes have largely replaced grassland over the last century (Archer et al., 1988; Boutton et al., 1998). Four of the most dominant woody species in this region (*Prosopis glandulosa* [Torr.] var. *glandulosa*, *Acacia rigidula* Benth., *Acacia farnesiana* (L.) Willd., and *Acacia berlandieri* Benth.) are known to be capable of symbiotic nitrogen fixation (Johnson and Mayeux ,1990; Zitzer et al.,1996). Many arid/semi-arid plant communities generally exhibit low productivity due to the constraints of water stress and N limitation (Rundel et al., 1982; Hooper and Johnson, 1999). Therefore, the establishment of these N-fixing tree species in grasslands have strong potential for altering the N-cycle, primary production, and other key ecosystem processes (Bush and Van Auken, 1986; Schlesinger et al., 1990; Stock et al., 1995; Pugnaire et al., 1996).

Plant species are likely to influence ecosystem N cycling via alterations in N use efficiency and by changing N inputs and losses (Knops et al., 2002). In southern Texas,

grassland-to-woodland conversion has resulted in increases in above- and belowground productivity in woodlands relative to adjacent remnant grasslands (Archer et al., 2001, 2004; Hibbard et al., 2001). The increased productivity resulting from grassland-to-woodland conversion influences both the quality and quantity of litter inputs to the soil. Alteration of litter quality and quantity in turn affect nutrient cycling processes. The increases in soil total N with woody plant invasion of grassland suggests that N transformations are affected by the establishment of these woody plant species in grasslands. Conversion of grassland to woodland has in fact resulted in changes to net mineralization and nitrification rates and to soil total N stocks in these affected areas in southern Texas (Hibbard et al., 2001; Archer et al., 2004; McCulley et al., 2004).

The natural abundance of soil ^{15}N is an integrator of N-cycling processes and is affected by both the biotic and abiotic environment (Robinson, 2001; Dawson et al., 2002). However, a complete understanding of the controls over patterns of $\delta^{15}N$ in the plant-soil system is still lacking. Globally, patterns of plant and soil $\delta^{15}N$ values are related to precipitation and temperature with $\delta^{15}N$ values decreasing with increasing mean annual precipitation and decreasing mean annual temperature (Handley et al., 1999; Amundson et al., 2003). Thus, arid regions are predicted to have the highest soil $\delta^{15}N$ values ranging from about 6.2-10.3 (Amundson et al., 2003). Locally, soil $\delta^{15}N$ values are generally more enriched than plant and litter $\delta^{15}N$, and tend to increase with depth in the soil profile; these trends reflect the cumulative effects of nitrogen isotope fractionation during decomposition and humification (Shearer et al., 1978; Nadelhoffer et al., 1996; Koba et al., 1998). However, soil $\delta^{15}N$ values are also influenced by a

number of factors such as quantity and quality of litter inputs, soil N sources, and isotopic fractionation resulting from N-transformations (Nadelhoffer and Fry, 1988; Piccolo et al., 1994).

Although multiple factors influence soil $\delta^{15}N$ values, alterations to the soil total N pool via a change in the balance of N inputs versus losses should be apparent in the natural abundance of ^{15}N in the plant-soil system. First, N-inputs from the atmosphere (N-fixation, wet and dry deposition) add N with low $\delta^{15}N$ values of approximately -3 to +3 ‰ (Amundson et al., 2003) and second, nearly all N-transformations lose ^{14}N-enriched N, leaving the product depleted and the residual N substrate relatively enriched in ^{15}N (Peoples et al., 1991; Hopkins et al., 1998; Robinson, 2001). Hence, changes in the $\delta^{15}N$ of soil total N should reflect the net result of input processes that generally deliver ^{15}N-depleted N to the system, and loss processes that generally leave the residual N pool enriched in ^{15}N.

The purpose of this study is to investigate the N-cycle consequences of grassland invasion by nitrogen-fixing tree legumes in a subtropical savanna parkland landscape using a chronosequence approach. More specifically, this study: (1) Quantified rates of N accumulation in litter, root biomass, and soil in woody plant stands differing in age, and (2) Utilized $\delta^{15}N$ values of litter, roots, and soil to provide qualitative insights into changes in N-cycling processes following grassland-to-woodland transitions.

MATERIALS AND METHODS

Study Area

Field sampling was conducted in December 2001 at the Texas Agricultural Experiment Station LaCopita Research Area (27^{o} 40' N; 98^{o} 12'W) located 65 km west of Corpus Christi, Texas in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. The climate is subtropical, with a mean annual temperature of 22.4^{o}C. Mean annual precipitation is 715 mm, and is bimodally distributed with peaks in May-June and September. The topography consists of nearly level uplands which grade (1-3%) into lower lying drainage woodlands. Elevation ranges from 75-90 m. This site has been grazed by domestic livestock over the past century.

Soils in uplands are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic horizon that includes non-argillic inclusions. Uplands are dominated by C_4 grasslands interspersed with small, discrete clusters of woody plants. Species of *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis* dominate the grasslands. The dominant plant species in all wooded landscape elements is *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite), a nitrogen-fixing tree legume (Johnson and Mayeux, 1990, Zitzer et al., 1996). Discrete clusters are characterized by a *Prosopis* overstory with species of *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory. Clusters expand laterally and fuse to form larger groves of vegetation where the argillic horizon is absent. Soils in lower-lying portions of the landscape are finer-textured loamy sands and clay loams (Pachic Argiustolls) and support closed-canopy woodlands. The vegetation composition of these

lower-lying drainage woodlands is similar to that in upland clusters and groves. Evidence from present vegetation patterns, sequential aerial photography, tree ring analyses, models of vegetation dynamics, and the isotopic composition of soils all indicate that this region was once relatively open grassland, and that woody plants have encroached into these grasslands over the past 150 years (Archer et al., 1988; Boutton et al., 1998; Archer et al., 2001, 2004). Additional details on plant communities and soils have been presented elsewhere (Scifres and Koerth, 1987; Archer et al., 1988; Boutton et al., 1998).

Chronosequence Approach

A chronosequence approach was used to quantify N pool sizes, their isotopic composition, and their rates of change in litter, roots, and soils following woody plant encroachment into areas that were previously grassland. Ten sites were sampled within remnant grasslands, clusters, and groves; eleven sites were sampled within drainage woodlands. All sites were located within an area of approximately 2 km^2. Remnant grasslands were sampled to characterize N pool sizes at time zero (i.e. prior to woody encroachment), whereas clusters, groves, and drainage woodlands of known age were sampled to evaluate changes in N pools at different points in time subsequent to woody invasion.

Ages of clusters, groves, and drainage woodlands were based on the fact that the formation of these wooded landscape elements is initiated only after the establishment of *Prosopis glandulosa* in grassland (Archer et al., 1988). Thus, the age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand. The ages of

P. glandulosa trees were determined by measuring their basal diameters, and then using those values to predict tree ages from regression equations specific to each landscape element (Stoker, 1997). Woody plant stands sampled in this study were selected to encompass the full range of *P.glandulosa* basal diameters at this site, corresponding to tree ages ranging from approximately 10-130 years.

Collection of Soil, Root, and Litter Samples

Surface litter was collected from a 0.25-m^2 quadrat at each site. In wooded landscape elements, the quadrat was located within 0.5 m of the bole of the largest *P. glandulosa* tree. Litter samples were washed with water over a 2-mm sieve to remove adhering soil particles, dried at 60EC to constant weight, weighed, pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI), and saved for elemental and isotopic analyses.

Four soil cores (5-cm diameter x 30-cm length) were taken within 0.5 m of the bole of the largest *P. glandulosa* at each wooded site, one in each cardinal direction from the bole. Sampling was identical in each remnant grassland site, but instead centered around the base of a large C_4 grass plant. Soil surface litter was removed gently to expose mineral soil prior to taking each soil core. All soil cores were sectioned into 0-15 and 15-30 cm increments and stored at 4^oC. In the lab, each soil sample was mixed thoroughly, and a subsample was dried at 105^oC to determine bulk density. Then, the 4 cores from each site were pooled by depth increment and mixed.

Prior to further treatment, an aliquot of each soil sample was set aside for determination of root biomass. Another aliquot was passed through a 2-mm sieve to

remove large organic fragments, and used for physical, chemical, and isotopic analyses. Soil pH was determined on a 1:2 (soil:water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipet method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was dried at 60^{o}C, pulverized in a centrifugal mill, and used for elemental and isotopic analyses.

Root biomass was quantified on well-mixed, aliquots (100 g) from each pooled soil sample using a hydropneumatic elutriation system (Smucker et al., 1982) (Gillison's Variety Fabrication, Inc., Benzonia, MI) equipped with a 410-µm screen. Roots were then dried at 60^{o}C, weighed, pulverized with a mortar and pestle, and saved for elemental and isotopic analyses.

Elemental and Isotopic Analyses

Litter, root, and soil samples were analyzed for C and N concentrations and for $\delta^{15}N$ values using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (ThermoFinnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Nitrogen isotope ratios are presented in δ-notation:

$$\delta^{15}N = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^{3} \quad (3)$$

where R_{SAMPLE} is the $^{15}N/^{14}N$ ratio of the sample, and R_{STD} is the $^{15}N/^{14}N$ ratio of the atmospheric N standard (Mariotti, 1983). Precision of duplicate measurements was <0.15 ‰.

Statistical Analyses

ANOVA was used to test for differences in soil physical and chemical characteristics with respect to landscape element and soil depth. ANOVA was also used

to test for differences in litter, root, and soil N stocks (g N m^{-2}) and $\delta^{15}N$ values with respect to landscape element and soil depth. Fisher's LSD method was used to identify significant differences among measurements due to the main effect of landscape element within each depth increment following ANOVA. Regression analyses were performed using Sigma Plot 4.0 (SPSS Inc., 1997) to determine the significance of relationships between woody plant stand age and N pool sizes in litter, roots, and soil.

RESULTS

Soil Characterization

Soil pH was 6.5 in the upper 15 cm of the profile in remnant grasslands, and was not altered following woody plant encroachment (Table 3). Bulk density of the 0-15 cm depth interval decreased from 1.2 g cm^{-3} in grasslands to 1.0 g cm^{-3} in wooded landscape elements (clusters, grove, drainage woodlands); at 15-30 cm, bulk densities ranged from 1.2-1.3 g cm^{-3}, but differed only between grassland and drainage woodland. Upland soils beneath grasslands, clusters, and groves were loamy sands, with a particle size distribution of 80% sand, 10% silt, and 10% clay. In contrast, soils in the lower-lying drainage woodlands were sandy loams comprised of 60% sand, 20% silt, and 20% clay. Soil organic carbon at 0-15 cm increased from 6 g C kg^{-1} soil in remnant grasslands to 11-21 g C kg^{-1} soil in wooded landscape elements; at 15-30 cm, soil organic carbon was 5 g C kg^{-1} soil in grasslands and 5-15 g C kg^{-1} soil in wooded areas (Table 3). Soil C/N ratios ranged from 10 to 13.

Table 3. Soil physical and chemical characteristics of grassland and woodland landscape elements at LaCopita Research Area. Fisher's LSD was used to separate differences between landscape elements within a depth interval following ANOVA. Different letters represent significant differences between means within a row for each depth interval. Standard errors of the mean are in parentheses.

	0-15 cm				15-30 cm			
	Grassland	**Cluster**	**Grove**	**Drainage**	**Grassland**	**Cluster**	**Grove**	**Drainage**
pH	6.5 (0.1)^{a}	6.4 (0.06)^{a}	6.3 (0.1)^{a}	6.2 (0.1)^{a}	6.9 (0.06)^{a}	6.4 (0.06)^{b}	6.7(0.05)^{a}	6.3 (0.1)^{b}
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Clay Loam
Sand (%)	80.5 (0.40)^{a}	81.3 (0.5)^{a}	81.4 (0.6)^{a}	66.0 (3.0)^{b}	74.1 (1.5)^{a}	78.8 (0.9)^{a}	78.0 (0.8)^{a}	56.1 (4.1)^{b}
Silt (%)	10.5 (2.0)^{ab}	9.6 (1.6)^{b}	12.0 (1.3)^{ab}	15.4 (2.7)^{a}	16.9 (1.9)^{ab}	12.5 (2.2)^{ab}	8.9 (2.2)^{c}	21.0 (2.1)^{a}
Clay (%)	9.0 (2.0)^{b}	9.1 (1.6)^{b}	6.6 (1.3)^{b}	18.6 (1.9)^{a}	9.1 (2.3)^{b}	8.7 (2.0)^{b}	13.1 (1.8)^{b}	22.9 (2.8)^{a}
Bulk Density ($g\ cm^{3}$)	1.2 (0.02)^{a}	1.0 (0.03)^{b}	1.0 (0.02)^{b}	1.0 (0.04)^{b}	1.3 (0.03)^{a}	1.3 (0.02)^{ab}	1.2 (0.02)^{ab}	1.2 (0.02)^{b}
SOC ($g\ C\ kg^{-1}$ soil)	6.2 (0.3)^{c}	11.2 (1.3)^{bc}	13.5 (1.6)^{b}	21.1 (3.1)^{a}	5.0 (0.4)^{b}	4.5 (0.3)^{b}	5.7 (0.3)^{b}	11.9 (1.3)^{a}
C/N	11.3 (0.2)^{ab}	10.8 (0.2)^{b}	11.3 (0.3)^{ab}	11.6 (0.2)^{a}	11.1 (0.4)^{b}	10.4 (0.2)^{c}	10.4 (0.2)^{c}	13.3 (0.4)^{a}

N-storage in Litter, Roots, and Soils Following Grassland-to-Woodland Conversion

On average, N stocks in surface litter in wooded areas (6-14 g N m^{-2}) were 200 to 600% greater than that in grassland litter (2 g N m^{-2}) (Fig. 7). Nitrogen stocks in root biomass at 0-15 cm in wooded areas (35-50 g N m^{-2}) were significantly greater (600 to 900%) than N in grassland root biomass (5 g N m^{-2}). At 15-30 cm, N stocks in root biomass in wooded areas (15-30 g N m^{-2}) were 200 to 500 % greater than N stocks in grassland roots (5 g N m^{-2}). Soil total N in the 0-15 cm depth increment was 50 to 150% greater in soils from wooded areas (150-250 g N kg^{-1} soil) than in soil from remnant grasslands (100 g N kg^{-1} soil at 0-15 cm); differences in soil N between grasslands and wooded areas were less pronounced at 15-30 cm (Fig. 7).

Pool sizes of N (g N m^{-2}) in litter, roots, and soil increased linearly over the past 100-130 years on those portions of the landscape where woody plants have encroached into grassland (Fig. 8). Woody plant stand age accounted for a significant proportion of the variation (approximately 30-80%) in the N stocks in litter, and in both roots and soils in the upper 15 cm of the profile. Accumulation rates for N in litter, roots, and soil were derived from the slopes of the linear regression fit to the data in Fig. 8 (Table 4). Nitrogen accumulation rates in whole soil to a depth of 30 cm ranged from 0.75-3.50 g N m^{-2} yr^{-1}. Most of the accumulation (75-83 %) was in the upper 15 cm of the soil profile. Nitrogen accumulation in litter accounted for only 2-6% of N accumulation rates in the soil system. In contrast to litter, accumulation rates of N in roots comprised 20-40 % of the N accumulation rates in whole soil (0-30 cm). Overall accumulation

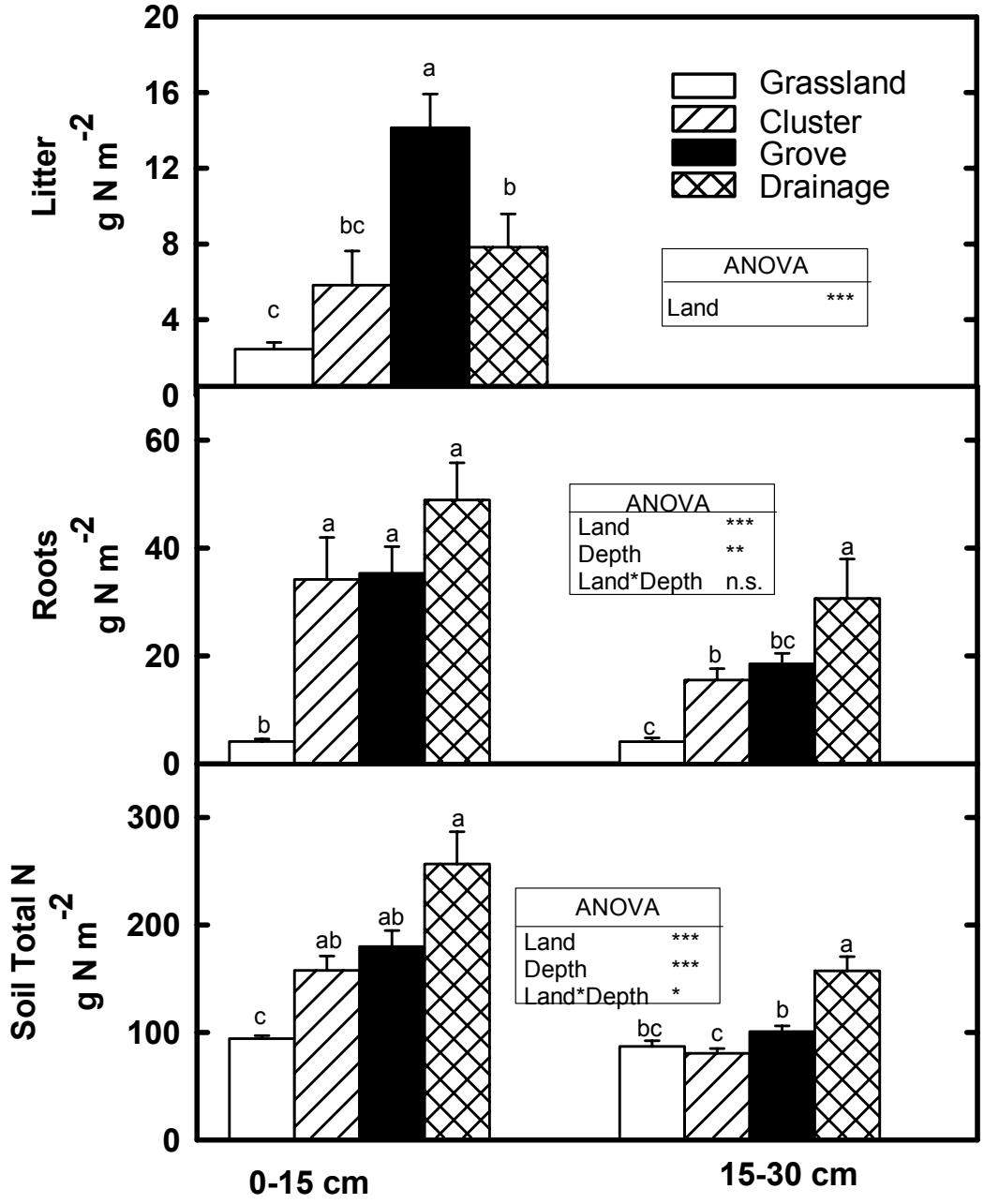

Fig. 7. Nitrogen densities (g N m^{-2}) in litter, roots, and soil in different landscape elements (grassland, cluster, grove, drainage woodland) in a subtropical savanna parkland at LaCopita Research Area by soil depth (0-15 and 15-30 cm) (n = 10 for grasslands, clusters, and groves; n = 11 for drainage woodlands). Error bars are standard errors of the mean. Asterisks denote level of significance from ANOVA ($p<0.05$*, $p<0.01$**, $p<0.001$***, n.s. = not significant at $p<0.05$). Different letters above bars indicate differences between means within a depth from Fisher's LSD.

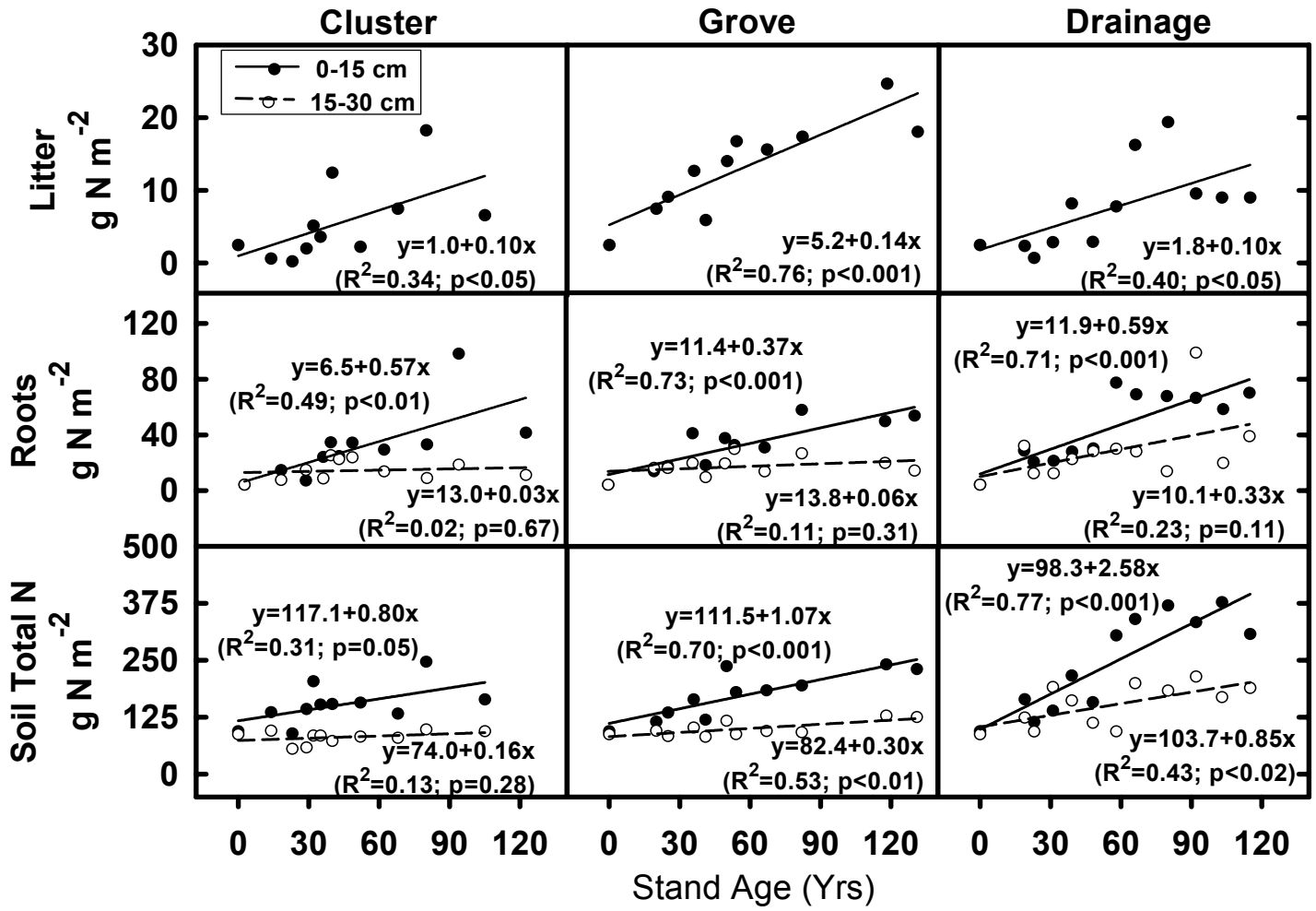

Fig. 8. Nitrogen densities (g N m^{-2}) in litter, roots, and soil relative to woody plant stand age in different landscape elements (cluster, grove, drainage woodland) in a subtropical savanna parkland. In each frame, time zero represents values for remnant grasslands.

Table 4. Accumulation rates of N in litter, roots, soil, and the total soil system (litter+roots+soil). Rates of litter, roots, and soil are derived from the slopes of the regression lines in Fig. 8. Rates from 0-30 cm for the total soil system were obtained by performing separate regressions on summed data. Asterisks denote significance of the linear regression at $p<0.05$.

	Accumulation Rates (g N m^{-2} yr^{-1})							
	Litter	Roots			Soil			Total
		0-15	15-30	0-30	0-15	15-30	0-30	
Cluster	0.10*	0.57*	0.03	0.63*	0.80	0.16	0.75*	1.58*
Grove	0.14*	0.37*	0.06	0.61*	1.07*	0.30*	1.43*	2.15*
Drainage	0.10*	0.59*	0.33	0.98*	2.58*	0.85*	3.50*	4.64*

rates for the entire soil system (litter + roots + soil) ranged from 1.58 g N m^{-2} in upland clusters to 4.64 g N m^{-2} in lower-lying drainage woodlands.

Changes in Soil $\delta^{15}N$ Following Grassland-to-Woodland Conversion

On average, $\delta^{15}N$ values of surface litter were not significantly different among landscape elements, ranging from 2.0-2.5‰ (Fig. 9). However, $\delta^{15}N$ values of roots in surface soils (0-15 cm) of wooded areas (2.0-2.4 ‰) were significantly lower than those in grasslands (3.2 ‰). In the 15-30 cm depth increment, $\delta^{15}N$ values of roots in both grasslands (2.1 ‰) and woodlands (0.6 ‰) were more depleted than those in surface soils. $\delta^{15}N$ values of soil total N at 0-15 cm were significantly higher in grasslands (7.5 ‰) than in all three wooded areas (6.0-6.4 ‰). This pattern was similar at 15-30 cm, but $\delta^{15}N$ values at this depth were more enriched than surface soil values by 1-2 ‰. $\delta^{15}N$ values of soil total N in the 0-15 cm depth increment decreased with time following woody plant encroachment from 7.5‰ in remnant grasslands to approximately 6‰ in clusters, groves, and drainage woodlands (Fig. 10). This 1.5‰ decrease occurred largely within the first 60 years of woodland development, and $\delta^{15}N$ values remained relatively constant from 60-120 years following woody plant encroachment. At 15-30 cm, whole-soil $\delta^{15}N$ values decreased over time following woody encroachment from 8.9‰ in remnant grasslands to 8‰ in clusters, and to 7‰ in groves and drainage woodlands. $\delta^{15}N$ values of litter and roots changed little over time following woody plant establishment in grassland, and their relationships with woody plant stand age were not significant.

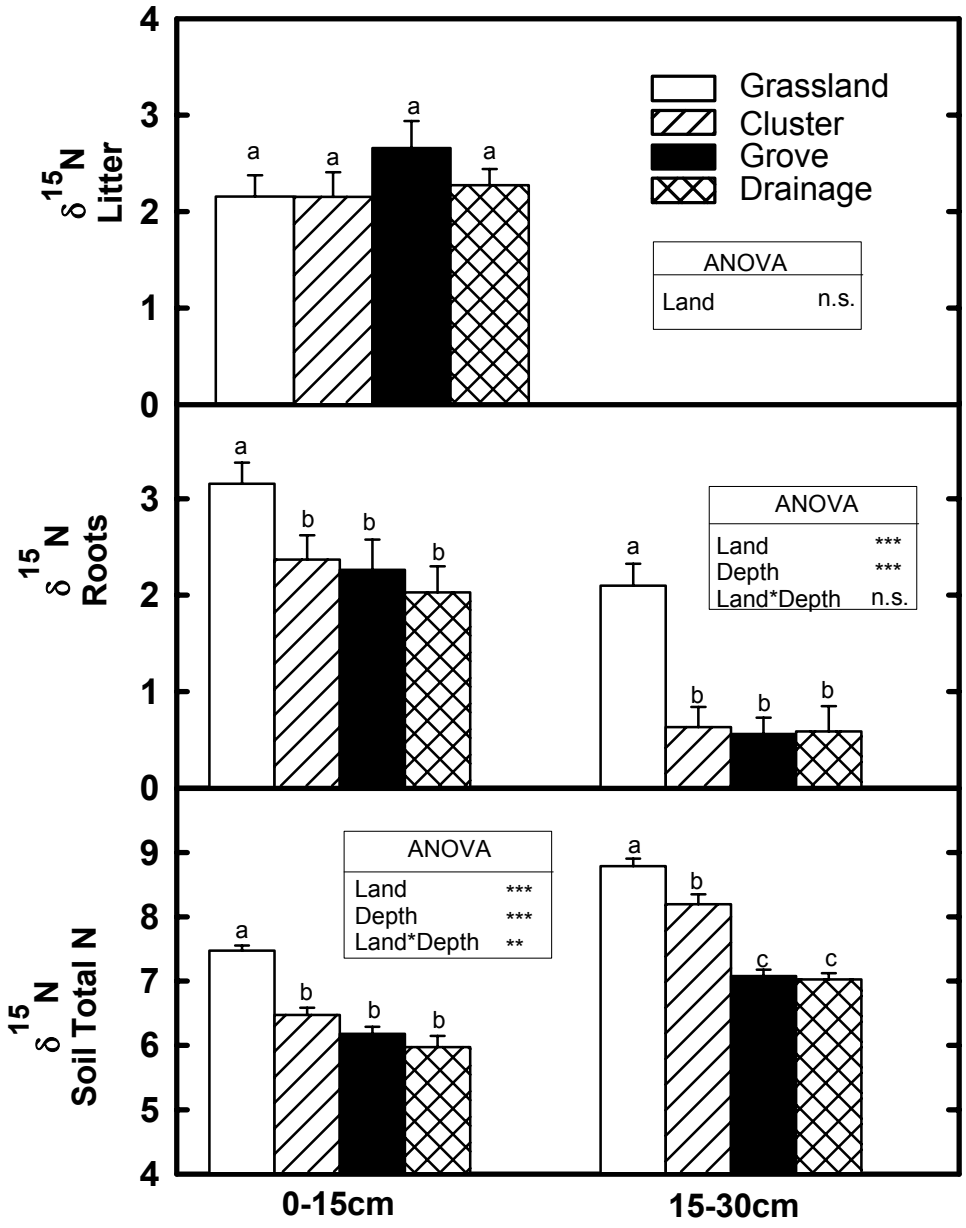

Fig. 9. $\delta^{15}N$ values of litter, roots, and soil total N in different landscape elements (grassland, cluster, grove, drainage woodland) in a subtropical savanna parkland. Error bars are standard errors of the mean. Asterisks denote level of significance from ANOVA ($p<0.05$*, $p<0.01$**, $p<0.001$***, n.s. = not significant at $p<0.05$). Different letters above bars represent significant differences among means within a depth from Fisher's LSD.

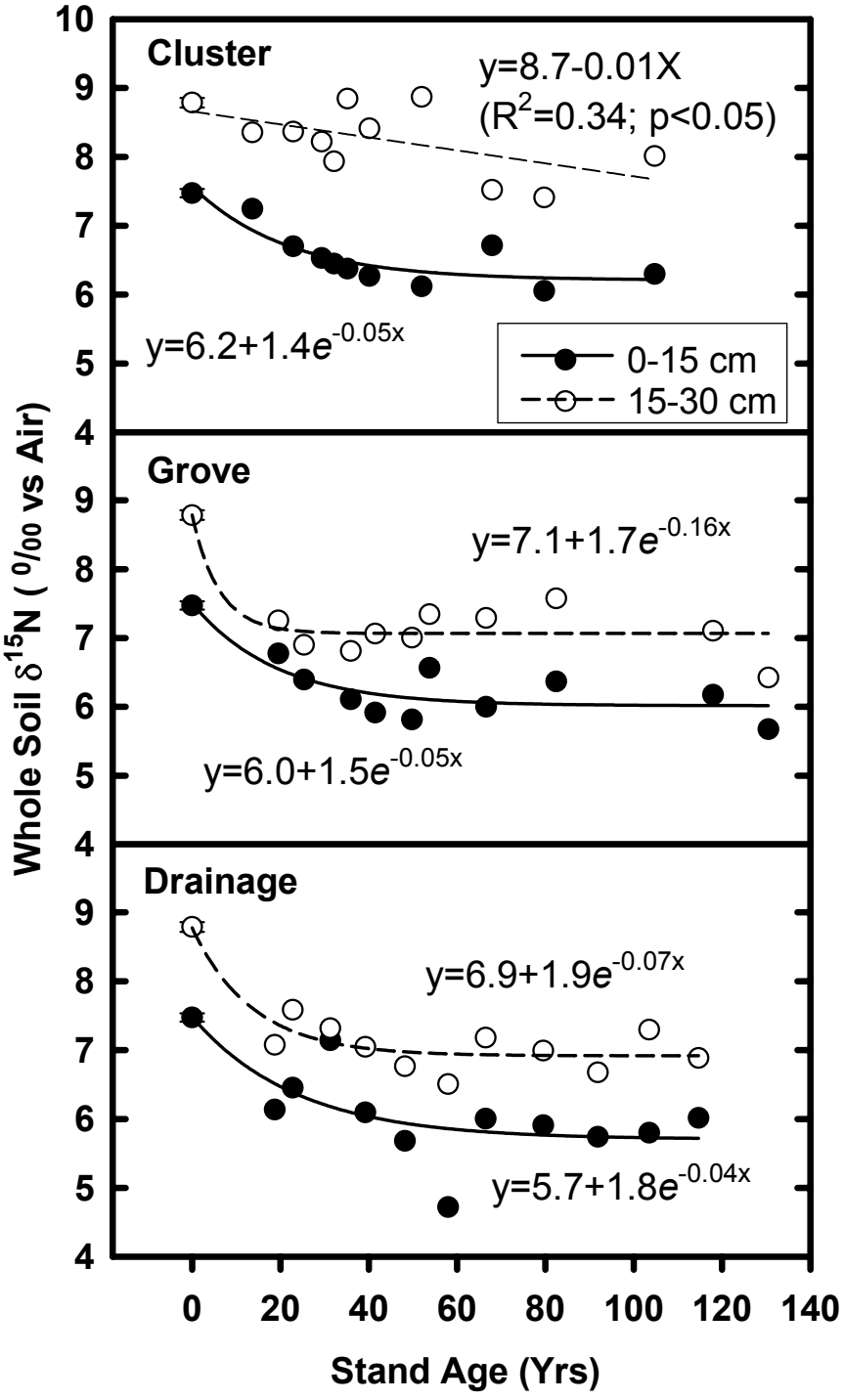

Fig. 10. Changes in δ^{15}N values of soil total nitrogen with respect to soil depth and woody plant stand age in a subtropical savanna parkland. Time zero in each frame is the δ^{15}N value of soil total nitrogen in remnant grasslands.

DISCUSSION

Nitrogen storage in litter, roots, and soil have increased linearly over the past 130 years following woody plant invasion of grassland in the Rio Grande Plains of southern Texas. Aboveground NPP has increased 50-200% from 1.9-3.4 Mg ha^{-1} yr^{-1} in remnant grasslands, to 5.1-6.0 Mg ha^{-1} yr^{-1} in areas now dominated by woody vegetation (Archer et al., 2001). Fine and coarse root biomass to a depth of 10 cm is up to 1000 % greater in wooded areas than in remnant grasslands, suggesting that belowground production is also accelerated significantly in woodlands (Hibbard et al., 2001). These significant increases in above- and belowground productivity have contributed to increased N storage in the soil system following woody plant encroachment into areas that were once grassland.

Soil total N to a depth of 30 cm in the soil increased 30-130% following grassland-to-woodland conversion (Fig. 7). These increases in soil total N (0-30 cm) beneath mesquite are comparable to the 30-100% increases found in other dryland ecosystems experiencing woody plant encroachment (Tiedemann and Klemmedson, 1973; East and Felker, 1993). Woodland soils (0-30 cm) in these areas in southern Texas are accumulating soil N at an average rate of 1.92 g N m^{-2} yr^{-1}. This rate is comparable to those estimated for woodland soils in the upper 20 cm of the profile by simulation modeling using CENTURY (0.6-1.2 g N m^{-2} yr^{-1}) and by previous field studies (1.90-4.64 g N m^{-2} yr^{-1}) at this same site (Archer et al., 2001, 2004). The average N accumulation rates for woodland soils in this study is comparable to estimated N

accumulation rates of 1-2 g N m^{-2} yr^{-1} for soils in other dryland ecosystems experiencing woody plant encroachment (Rundel et al., 1982; Geesing et al., 2000).

In addition to higher inputs of N from the increased above- and belowground productivity following woody plant invasion of grassland, it is probable that soil texture and soil moisture also contribute to observed patterns of soil N accumulation. Highest soil N accumulations occurred in drainage woodlands, perhaps as a result of the higher silt and clay content which can stabilize organic matter and protect it from decay, and/or higher rates of NPP due to more favorable soil moisture regimes in this lower-lying portion of the landscape. In contrast, the lower N accumulations in clusters and groves are likely due to the coarser soil textures in the uplands. Similarly, in a Sonoran Desert ecosystem, highest N accumulations occurred in soils beneath mesquite on lower elevational sites with high clay content while lower accumulations of soil total N were observed beneath mesquite occurring on sandy dunes (Virginia and Jarrell, 1983).

The significant increases in soil total N in this dryland region in southern Texas suggest that N inputs have exceeded outputs resulting in net N accumulation over the past century following woody invasion of grassland. Alternatively, N-inputs could remain unchanged following woody encroachment, but N-losses might be diminished in wooded areas. This might occur if plant uptake and/or microbial immobilization of NH_4^+ and NO_3^- were elevated in wooded areas compared to remnant grasslands, thereby reducing the potential for those inorganic N species to serve as substrate for N-losing processes such as nitrification and denitrification.

The observation that root biomass (Boutton et al., 1998, 1999; Hibbard et al., 2001) and soil microbial biomass (McCulley et al., 2004) are both significantly greater in wooded areas than in remnant grasslands indicates that increased immobilization of inorganic N by microbes and uptake by woody plants is a plausible hypothesis. In a study of the root decomposition of tropical forage species, roots of leguminous species were more highly degradable than grass species resulting in greater immobilization of soil mineral N by soil microbes (Urquiaga et al., 1998). However, prior studies have confirmed that N losses have increased following woody plant invasion of grassland. Increased N mineralization rates, nitrification, and trace gas fluxes from woodland soils reflect an acceleration of N-cycling processes and higher losses of N following woody plant invasion of grassland (Cole et al., 1996; Hibbard et al., 2001; McCulley et al., 2004). These studies suggest that N losses have not diminished following woody plant invasion.

Nevertheless, despite higher losses of N from woodlands relative to remnant grasslands, N has accumulated in soils over the past century. Evidence from the natural abundance of ^{15}N supports the hypothesis that inputs have exceeded losses. Because ecosystem N inputs via atmospheric deposition ($\delta^{15}N$ = -3 to +3 ‰) and N_2-fixation ($\delta^{15}N$ = -2 to +1 ‰) are generally ^{15}N-depleted, and because gaseous N-losses via microbial transformations (NH_3, NO_x, N_2O, N_2) are also ^{15}N-depleted ($\delta^{15}N$ = -40 to 0 ‰), changes in inputs and/or loss rates can alter the $\delta^{15}N$ of the soil total N pool (Hogberg, 1997; Handley et al., 1999; Robinson, 2001). When inputs exceed losses, the soil total N pool should become ^{15}N-depleted (i.e. lower $\delta^{15}N$); conversely, when losses

exceed inputs, the soil total N pool should become ^{15}N-enriched (i.e. higher δ^{15}N) due to loss of ^{15}N-depleted gaseous N. Soil total N cannot become depleted or enriched in ^{15}N without the transfer of N into or out of the soil (Handley and Raven, 1992).

δ^{15}N values of N from litter and root biomass were more depleted than soil total N across all landscape elements, and δ^{15}N values of soil total N became enriched with depth in the profile. The addition of ^{15}N-depleted N through litterfall and root turnover may be an explanation for the lower δ^{15}N values in surface soils compared to soil at deeper depths (Nadelhoffer and Fry, 1988; Hogberg, 1997). In addition, it is generally accepted that the pattern of increasing soil δ^{15}N values with increasing depth in the profile is a result of the increasing age and decomposition of organic matter over time (Nadelhoffer and Fry, 1988; Shearer and Kohl, 1989; Gebauer and Schulze, 1991). Whole soil δ^{15}N values (6-7.5 ‰) in this subtropical savanna parkland in southern Texas were within the range of values (6-10 ‰) estimated for arid regions based on precipitation and temperature (Handley et al., 1999; Amundson et al, 2003). High soil δ^{15}N values for arid and semiarid ecosystems indicate that these systems have relatively open N-cycles with high losses relative to inputs.

Soil δ^{15}N values at both 0-15 and 15-30 cm depth increments became more depleted with time following woody plant encroachment, suggesting that N-inputs exceed N-losses during woodland development. This hypothesis is consistent with elevated soil N concentrations and densities observed in wooded portions of the landscape. One potential mechanism for increased soil N in woodlands is increased inputs from litter and roots. Although litter inputs of both grassland and woodland have

low $\delta^{15}N$ values, they are not significantly different. In contrast, $\delta^{15}N$ values of roots in woodlands (2 ‰) are significantly lower than roots in grasslands (3 ‰), suggesting that the decline in soil $\delta^{15}N$ values observed following woody plant invasion of grassland might be due to increased inputs of ^{15}N-depleted roots.

One mechanism by which roots may be contributing to increased soil N accumulation in surface soils is through mining of deeper soil N by the more deeply rooted wooded plant species. A prior study showed that root biomass of woody plants was significantly greater throughout the soil profile to depths >5 m whereas grasslands had almost no root biomass below 1.5m in the soil profile (Boutton et al., 1999). The greater root biomass throughout the entire soil profile allows woody plants to explore and mine N from a much greater volume of soil. It has been suggested that redistribution of soil N from deeper to shallower soil depths can be an important consequence when deep-rooted plants replace shallow-rooted plants during succession (Knops and Tilman, 2000). However, based on isotopic evidence, this mechanism seems unlikely as N from deeper in the soil tends to have increased $\delta^{15}N$ values and soils are becoming increasingly depleted with time following woody plant invasion of grassland.

Inputs from symbiotic N-fixation are a potential source of isotopically light N inputs ($\delta^{15}N$ = -2 to 1 ‰) to soil. *Prosopis glandulosa* (the dominant woody plant in clusters, groves, and drainage woodlands) is well known for its ability to fix atmospheric N (Johnson and Mayeux, 1990), and has been shown to develop active root nodules when grown in soils from this study area (Zitzer et al., 1996). In addition, 6 other tree-

legume species at this site form active root nodules and likely fix atmospheric N under field conditions. In a Sonoran desert ecosystem in Arizona, large amounts of N accumulated in the surface 0-30 cm beneath mesquite (Virginia and Jarrell, 1983). The increases in soil total N were attributed largely to N-fixation. Others have reported similar dilutions of soil $\delta^{15}N$ values by fixed N derived from N-fixing tree species (Shearer and Kohl, 1989; Peoples et al., 1991; Stock et al., 1995). Estimates of 1-16 g N m^{-2} yr^{-1} have been reported for contributions by N-fixing species present in early successional stages (Boring et al., 1988). These estimates suggest that 100% of the observed N accumulation in soils and in the soil system (litter + roots + soil) in this subtropical ecosystem could be due to inputs from biological N-fixation. Thus, inputs of biologically fixed N may be responsible for the decrease in soil $\delta^{15}N$ values following woody plant encroachment into grasslands. Other potential sources of biologically fixed N are free-living cyanobacteria and termites. Estimated inputs of N from non-symbiotic N-fixation range from 0.1-0.5 g N m^{-2} yr^{-1} (Boring et al., 1988). Termites may be a more probable source of isotopically light N inputs to soil since termites are major consumers of woody detritus in mulga and other arid vegetation communities (Pate et al., 1998). Substantial termite activity has been observed in this savanna parkland (personal observation).

Another plausible mechanism for increased N inputs in woodland soils is atmospheric deposition. The greater canopy height and leaf area in woodlands vs. grasslands may be more effective in capturing and trapping atmospheric N-deposition, resulting in greater accumulation of this source in wooded areas. The isotopic

composition of N derived from atmospheric deposition is generally depleted, yielding negative values for both ammonium and nitrate (Vitousek et al., 1989). Since atmospheric deposition was not measured, it is not possible to determine the total contribution of N from atmospheric sources to soil total N accumulation following grassland-to-woodland conversion. However, the National Atmospheric Deposition Program (2004) reports wet deposition of 0.2-0.4 g N m^{-2} yr^{-1} for a region near the study site, and dry deposition may increase that amount. Estimated rates of N input from bulk atmospheric deposition (wet and dry) across a diverse range of non-agricultural ecosystems were 0.1-1.2 g N m^{-2} yr^{-1} (Boring et al., 1988).

CONCLUSIONS

Increased inputs of N relative to losses have resulted in net sequestration of N in soils of a subtropical savanna ecosystem in the Rio Grande Plains of southern Texas following woody plant invasion of grassland. Rates of N accumulation in woodland soils have been linear over the past 100 years, and show no signs of departing from linearity at present. These increases of N in litter, roots, and soil following woody plant invasion of grassland are likely due to increased above- and belowground productivity of woody plants relative to the original grassland, and to symbiotic nitrogen fixation by the dominant woody plants. Soil texture may have influenced patterns of soil total N storage in this dryland ecosystem, with higher N accumulations associated with lower lying drainage woodland soils with increased silt and clay content. Soil $\delta^{15}N$ showed enrichment with depth consistent with the pattern of increasing $\delta^{15}N$ values with

increasing magnitude of decomposition. Decreasing soil $\delta^{15}N$ values with increasing woody plant stand age suggests that N accumulation has occurred as a result of N inputs exceeding losses over the past century. Inputs of isotopically light N from N-fixation and atmospheric deposition are potential N sources that could cause depletion of the soil ^{15}N pool. Evidence from the natural abundance of ^{15}N in conjunction with evidence of root nodulation in the dominant woody plant species suggests that N-fixation is a major source of N in these *Prosopis* dominated woodlands.

CHAPTER IV

SOIL MICROBIAL BIOMASS RESPONSE TO WOODY PLANT INVASION OF GRASSLAND

INTRODUCTION

Worldwide increases in woody plant proliferation in grassland and savanna ecosystems have been documented in recent history (Archer et al., 2001). This geographically extensive phenomenon is attributable to human land-use activities, primarily livestock grazing and fire suppression (Archer et al., 2001). Because plant species exert strong controls on soil organic matter dynamics via the quantity and quality of litter inputs to soil (Scholes et al., 1997; Chapin et al., 2002), land-use and land cover changes which result in restructuring of the plant community have strong potential to modify key ecosystem functions including the C cycle.

The size and dynamics of the soil organic matter pool depend to a large extent on the quantity and quality of C inputs to the soil (Collins et al., 1997). Levels of soil organic carbon (SOC) are the result of the balance between above- and belowground C inputs to the soil and the loss of C via decomposition (Schlesinger, 1997; Amundson, 2001). Changes in the magnitude of the SOC pool over time are often difficult to detect because of the large background and natural variability of the organic matter already present in the soil (Powlson et al., 1987; Scholes et al., 1997).

Approaches based on the characterization of active soil organic matter pools with more rapid turnover rates have been suggested as a more effective measure of changes in SOC (Sparling, 1992). Turnover times of SOC are comparatively slow (10s to 1000s of

yrs) relative to turnover of the soil microbial biomass (days), which play a critical role in regulating nutrient storage and fluxes (Horwath and Paul, 1994; Dalal, 1998). Thus, soil microbial biomass has been utilized as a sensitive indicator of the effects of changes in the quality and quantity of organic matter inputs on soil organic matter levels as a result of changes in land management or land-use practices, especially over shorter time intervals (Powlson et al., 1987; Anderson and Domsch, 1989). Additionally, changes in C_{mic}/C_{org} (the ratio of soil microbial biomass C (SMB-C) to total SOC) and the metabolic quotient or qCO_2 (unit CO_2-C unit^{-1} C_{mic} hr^{-1}) have been utilized to reflect changes in organic matter input to soils, microbial efficiency in converting available C to SMB-C, C losses from soil, and the stabilization of organic C by soil mineral fractions (Sparling, 1992).

In the Rio Grande Plains of southern Texas, subtropical woodlands have largely replaced grassland over the last century primarily as a result of continuous, heavy livestock grazing (Archer et al., 1988; Boutton et al., 1998). Four of the most dominant woody species in these invasive woodlands (*Prosopis glandulosa* [Torr.] var. *glandulosa*, *Acacia rigidula* Benth., *Acacia farnesiana* (L.) Willd., and *Acacia berlandieri* Benth.) are known N-fixing species (Johnson and Mayeux, 1990; Zitzer et al.,1996). Hence, the establishment of these N-fixing tree species in N-limited grasslands has strong potential to affect primary productivity and ecosystem nutrient cycling. Documented increases in above- and belowground productivity in wooded areas vs. remnant grasslands in this region (Archer et al., 2001; Hibbard et al., 2001) suggest that woody encroachment has affected C storage and transformations in this

semi-arid ecosystem. However, the mechanism(s) of C sequestration and the quality of the organic matter that has accumulated is largely unknown.

To quantify changes in SOC storage and dynamics following woody plant invasion of grassland in the Rio Grande Plains of southern Texas, I collected soils from chronosequences consisting of remnant grassland which represented time before woody invasion (Time 0) and from woody plant stands ranging in age from 10-130 years in upland and lowland landscape elements. I hypothesized that: (1) SOC and soil total nitrogen (STN) would increase under woody plant stands relative to grassland; (2) This increase in C and N content would result in an increase in SMB-C; (3) The C_{mic}/C_{org} ratio would decrease with increasing woody plant stand age due to the more recalcitrant nature of lignified woody litter compared to herbaceous litter; and (4) The qCO_2 would increase with time after woody invasion suggesting lower microbial efficiency and/or that the woodland C is of poorer quality as also reflected by a lower C_{mic}/C_{org} ratio.

MATERIALS AND METHODS

Study Area

Soils were collected from the Texas Agricultural Experiment Station LaCopita Research Area (27° 40'N; 98° 12'W) located 65 km west of Corpus Christi, Texas in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical, with a mean annual precipitation of 715 mm (bimodally distributed) and a mean annual temperature of 22.4°C. Topography consists of nearly level uplands which grade (1-3%) into lower-lying drainage woodlands and playas. The elevation ranges from 75-90 m.

Originally classified as a *Prosopis-Acacia-Andropogon-Setaria* savanna (Kuchler, 1964), contemporary vegetation is subtropical thorn woodland, having experienced significant woody plant expansion in recent history. Over the past century, this area was heavily grazed by domestic livestock, which has been identified as a causal factor in succession from grassland to woodland (Archer et al., 1988).

Upland soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface (B_t) horizon with non-argillic inclusions, and are characterized by a two-phase vegetation pattern consisting of discrete clusters of woody vegetation embedded within a matrix of remnant C_4 grasses. Species of *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis* dominate in the grasslands. Formation of clusters is initiated when grasslands are colonized by *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite), which then facilitates recruitment of other woody plant species in the understory (Archer et al., 1988). Where the argillic horizon is absent, clusters expand laterally and fuse to form larger groves of woody vegetation (Archer et al., 1995). Soils in lower-lying drainage areas have clay loam soils (Pachic Argiustolls) and are characterized by continuous closed-canopy drainage woodlands. These drainage woodlands appear to have originated from the same successional processes currently underway in uplands, and their vegetation composition is similar to that in upland clusters and groves. *P. glandulosa* is the dominant species in all wooded landscape elements with *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory.

Chronosequence Approach

To quantify changes in soil characteristics following woody plant invasion of areas that were formerly grasslands, a chronosequence approach was utilized. Within upland landscape elements, 10 sites were sampled in remnant grasslands, clusters, and groves; 11 sites were sampled within lower-lying drainage woodlands. All sites were located within an area of approximately 2 km^2. To characterize SMB-C at time zero (i.e. prior to woody encroachment), soils were sampled in remnant grasslands. Clusters, groves, and drainage woodland landscape elements were sampled to evaluate changes in SMB-C at known points in time following woody plant encroachment.

The age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand since formation of these wooded landscape elements is initiated only after the establishment of *Prosopis glandulosa* in grassland (Archer et al., 1988). Therefore, ages of *P. glandulosa* trees were determined by measuring their basal diameters, and then using those values to predict tree ages based on regressions between basal diameter and tree age; equations specific to each wooded landscape element were developed previously (Stoker, 1997). In this study, the woody plant stands sampled were selected to encompass a wide range of *P.glandulosa* basal diameters and corresponded to tree ages ranging from approximately 10-130 years.

Collection and Analyses of Litter, Roots, and Soil

At each site, all surface litter within a 0.25-m^2 quadrat was collected. In wooded landscape elements, the quadrat was located within 0.5 m of the bole of the largest *P. glandulosa* tree. To remove adhering soil particles, litter samples were washed with

deionized water over a 2-mm sieve, oven-dried at 60^{o}C to constant weight, weighed, pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI), and retained for elemental analyses.

In wooded landscape elements, 4 soil cores (5-cm diameter x 30-cm depth) were taken beneath the largest *P. glandulosa*, one in each cardinal direction from the bole. Sampling in remnant grassland sites was identical, but cores were taken around the base of a large C_4 grass plant instead. Prior to soil coring, soil surface litter was gently removed to expose the mineral soil. Each soil core was sectioned into 2 depth increments (0-15 and 15-30 cm) and stored at 4^{o}C. In the lab, soils were thoroughly mixed to homogenize the samples. A subsample of each soil was dried at 105^{o}C to determine bulk density. The 4 soil cores from each site were pooled by depth increment and again thoroughly mixed.

An aliquot (approximately 25%) of each soil sample was set aside for determination of root biomass prior to subsequent analyses. For determination of physical and chemical analyses, another aliquot was passed through a 2-mm sieve to remove large organic fragments. Soil pH was determined on a 1:2 (soil:water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipet method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was oven-dried at 60^{o}C, pulverized in a centrifugal mill, and used for elemental analyses.

Root biomass was quantified on well-mixed, aliquots (100g) from each pooled soil sample using a hydropneumatic elutriation system (Smucker et al., 1982) (Gillison's Variety Fabrication, Inc., Benzonia, MI) equipped with a 410-µm screen. Roots were

then dried at 60^{o}C, weighed, pulverized with a mortar and pestle, and saved for elemental analyses. Carbon and nitrogen concentrations of litter, roots, and soil were determined on a Carlo Erba EA-1108 (Finnigan MAT, Bremen, Germany).

An additional subsample of soils was sieved <4 mm for determination of SMB-C. SMB-C was determined by the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976; Horwath and Paul, 1994). Results presented are without the subtraction of a control, since this method yields estimates of SMB-C that are more highly correlated with other soil variables such as potential C-mineralization, SOC, and SMB-C than those derived via subtraction of a control (Franzluebbers et al., 1999a,b).

Statistical Analyses

Regression analyses were performed using Sigma Plot 4.0 (SPSS Inc., 1997) to determine significance of relationships between SMB-C (controls not subtracted) and soil characteristics (SOC, STN, litter C and N, and root C and N). Regression analyses were also utilized to determine the significance of relationships between woody plant stand age and SMB-C, C_{mic} / C_{org}, and qCO_2. ANOVA (NCSS, 1995) was used to analyze soil physical and chemical characteristics, SMB-C, C_{mic}/C_{org}, and qCO_2 relative to landscape element and soil depth. Fisher's LSD method was used to identify significant differences among measurements due to the main effect of landscape element within each depth increment following ANOVA.

RESULTS

Soil Physical and Chemical Characteristics

Soil pH in the upper 0-15 cm of the soil profile was 6.5 in remnant grasslands and was not significantly altered following woody plant establishment in grasslands (Table 5). Bulk density (0-15 cm) decreased from 1.2 in remnant grasslands to 1.0 in wooded landscape elements (clusters, groves, and drainage woodlands). In upland clusters and groves, particle size distribution was approximately 80% sand, 10% silt, and 10% clay in surface soils (0-15 cm). In contrast, the particle size distribution of lower-lying drainage woodlands was 60% sand, 20% silt, and 20% clay (Table 5). SOC and STN in wooded landscape elements increased up to 250% relative to remnant grassland (Table 5). C/N ratios of whole soil (0-15 and 15-30cm depth increments) ranged from 10-13.

Changes in SMB-C Following Woody Plant Invasion of Grassland

SMB-C (mg C kg^{-1} soil) showed a trend towards higher values in woodland soils relative to remnant grassland across both soil depth increments (Fig. 11). SMB-C in drainage woodlands increased 75% relative to remnant grassland in the upper 15 cm of the profile. However, SMB-C in clusters and groves (0-15 cm) was not significantly greater than the remnant grassland value (Fig. 11). SMB-C estimates in all wooded landscape elements were significantly greater (300-400 %) than grassland SMB-C at the 15-30 cm depth (Fig. 11).

Relative to woody plant stand age, SMB-C in the surface 0-15 cm increased linearly from approximately 300 mg C kg^{-1} soil in woody plant stands < 30 years old up

Table 5. Summary of soil physical and chemical characteristics of grassland and woodland landscape elements at LaCopita Research Area. Fisher's LSD were used to separate differences between landscapes within a depth interval following ANOVA. Different letters represent significant differences between means within a row for each depth interval. Standard errors of the mean are in parentheses.

	0-15 cm				15-30 cm			
	Grassland	Cluster	Grove	Drainage	Grassland	Cluster	Grove	Drainage
pH	6.5 (0.1)^{a}	6.4 (0.06)^{a}	6.3 (0.1)^{a}	6.2 (0.1)^{a}	6.9 (0.06)^{a}	6.4 (0.06)^{b}	6.7(0.05)^{a}	6.3 (0.1)^{b}
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Clay Loam
Sand (%)	80.5 (0.40)^{a}	81.3 (0.5)^{a}	81.4 (0.6)^{a}	66.0 (3.0)^{b}	74.1 (1.5)^{a}	78.8 (0.9)^{a}	78.0 (0.8)^{a}	56.1 (4.1)^{b}
Silt (%)	10.5 (2.0)^{ab}	9.6 (1.6)^{b}	12.0 (1.3)^{ab}	15.4 (2.7)^{a}	16.9 (1.9)^{ab}	12.5 (2.2)^{ab}	8.9 (2.2)^{c}	21.0 (2.1)^{a}
Clay (%)	9.0 (2.0)^{b}	9.1 (1.6)^{b}	6.6 (1.3)^{b}	18.6 (1.9)^{a}	9.1 (2.3)^{b}	8.7 (2.0)^{b}	13.1 (1.8)^{b}	22.9 (2.8)^{a}
Bulk Density ($g\ cm^{-3}$)	1.2 (0.02)^{a}	1.0 (0.03)^{b}	1.0 (0.02)^{b}	1.0 (0.04)^{b}	1.3 (0.03)^{a}	1.3 (0.02)^{ab}	1.2 (0.02)^{ab}	1.2 (0.02)^{b}
SOC (%)	0.6 (0.02)^{c}	1.1 (0.1)^{bc}	1.4 (0.2)^{b}	2.1 (0.3)^{a}	0.5 (0.02)^{b}	0.4 (0.03)^{b}	0.6 (0.03)^{b}	1.2 (0.1)^{a}
$g\ C\ m^{-2}$	1050 (43)^{c}	1715 (172)^{bc}	2062 (207)^{b}	2981 (350)^{a}	994 (75)^{b}	838 (52)^{b}	1054 (54)^{b}	2121 (228)^{a}
Soil Total N (%)	0.05 (0.001)^{c}	0.10 (0.01)^{b}	0.11 (0.01)^{b}	0.18 (0.03)^{a}	0.05 (0.002)^{b}	0.04 (0.002)^{b}	0.05 (0.003)^{b}	0.09 (0.01)^{a}
$g\ N\ m^{-2}$	94.2 (2.8)^{c}	157.8 (13.4)^{b}	179.9 (14.9)^{b}	256.7 (30.0)^{a}	87.1 (5.1)^{b}	80.5 (4.6)^{b}	100.7 (5.4)^{b}	157.3 (13.2)^{a}
C/N	11.3 (0.2)^{ab}	10.8 (0.2)^{b}	11.3 (0.3)^{ab}	11.6 (0.2)^{a}	11.1 (0.4)^{b}	10.4 (0.2)^{c}	10.4 (0.2)^{c}	13.3 (0.4)^{a}

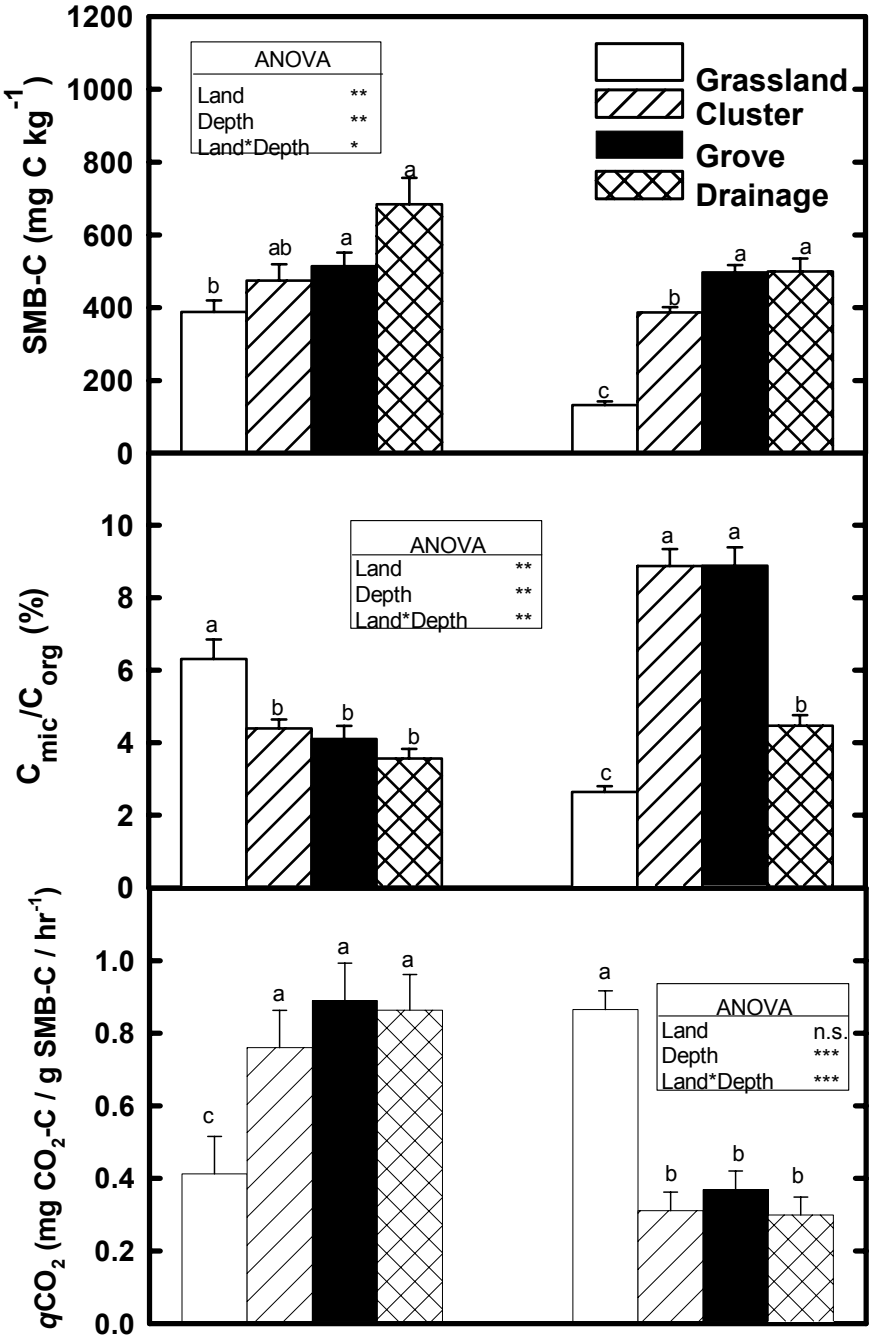

Fig.11. SMB-C, C_{mic}/C_{org}, and qCO_2 of soils in different landscape elements at LaCopita Research Area by soil depth (0-15 and 15-30 cm). Error bars are standard errors of the mean. Asterisks indicate degree of significance from ANOVA ($p<0.05$*, $p<0.01$**, $p<0.001$***). Different letters above bars indicate significant differences among means within a depth from Fisher's LSD.

to as high as 900 mg C kg $^{-1}$ soil in woody plant stands > 60 years (Fig. 12). SMB-C at 15-30 cm in the profile followed a similar pattern to SMB-C in surface soils, increasing from 100 mg C kg $^{-1}$ in younger woody plant stands (< 30 years) up to 500 mg C kg $^{-1}$ in older woodlands (> 60 years) (Fig. 12).

Relationships of SMB-C with Characteristics of Plant-Soil System

SMB-C in cluster and drainage woodland soils (0-15 cm) was significantly correlated with SOC, STN, litter C and N, and root C and N, accounting for 50-90% of the variation in the estimates (Table 6). In grove soils (0-15 cm), only SOC and STN were significantly correlated with SMB-C, accounting for 50-60% of the estimates. SMB-C in grassland soils (0-15 cm) was not significantly correlated with any of the measured characteristics (Table 6). At the 15-30 cm depth, correlations of SMB-C with characteristics of the plant-soil system were variable. In grasslands (15-30 cm) SMB-C was significantly correlated with SOC. In contrast, SMB-C in clusters at depth was significantly correlated with STN. In grove soils at 15-30 cm, SOC and STN were not significantly correlated with SMB-C although litter C and N and root C and N showed significant correlations. Drainage woodland SMB-C at depth was significantly correlated with both SOC and STN (Table 6). Estimates of SMB-C from grassland and woodland landscape elements with subtraction of a control were not significantly correlated with any of the quantified characteristics in Table 6 (data not shown).

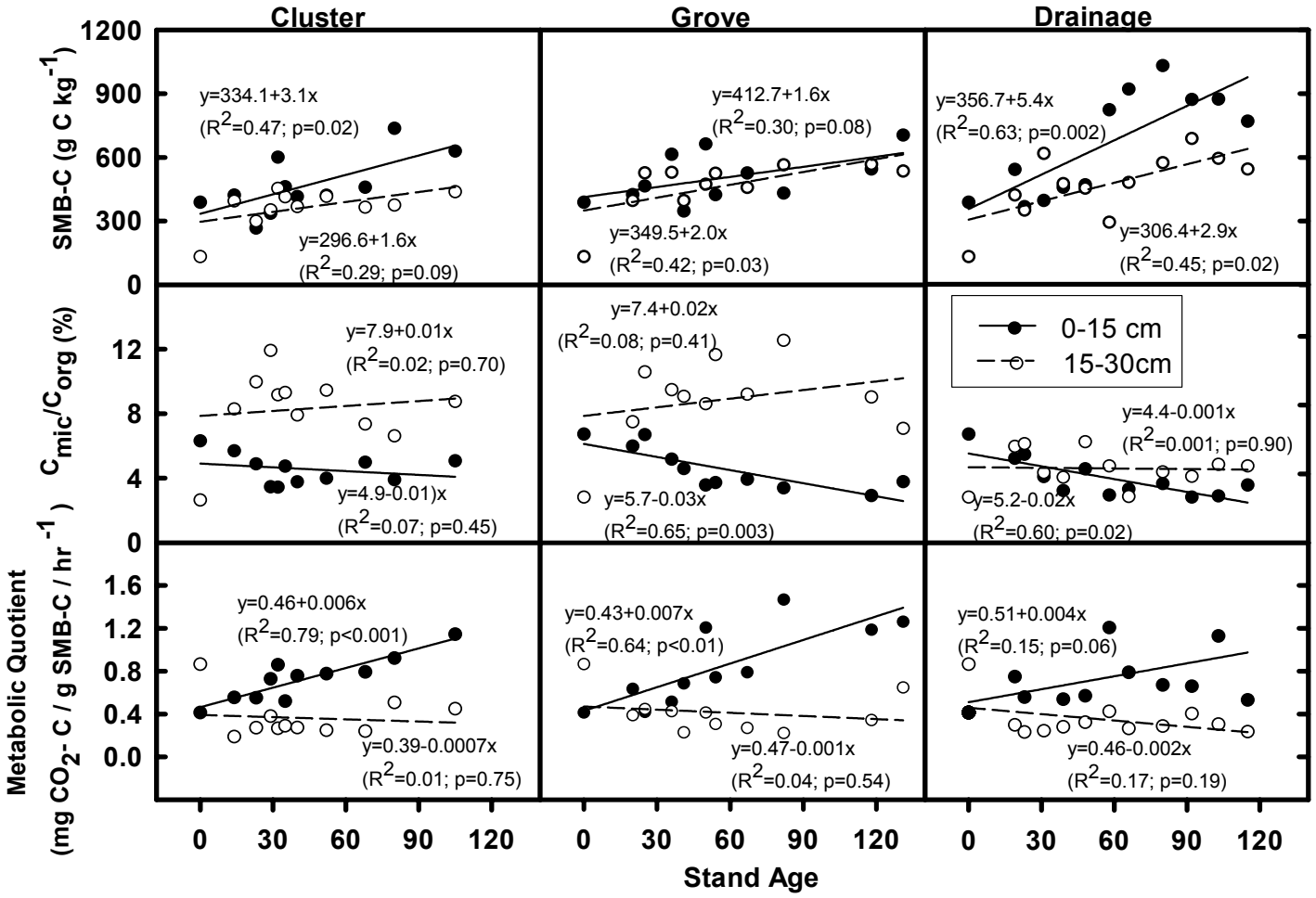

Fig. 12. Changes in SMB-C, C_{mic}/C_{org}, and qCO_2 in soils (0-15 cm and 15-30 cm) with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at Time 0 and represent baseline starting values.

Table 6. Correlations between SMB-C (controls not subtracted) and characteristics of plant-soil system (g m^{-2}). Values are correlation coefficients (r), and asterisks indicate degree of significance ($p<0.05$*, $p<0.01$**, $p<0.001$***).

	SMB-C							
	Grassland		Cluster		Grove		Drainage	
	0-15cm	15-30cm	0-15cm	15-30cm	0-15cm	15-30cm	0-15cm	15-30 cm
SOC	0.21	0.67*	0.89***	0.55	0.79**	0.26	0.95***	0.79**
STN	0.25	0.38	0.89***	0.69*	0.71*	0.38	0.96***	0.88***
Litter C	0.14	0.34	0.70*	0.11	0.58	0.78**	0.86***	0.38
Litter N	0.01	0.27	0.71*	0.18	0.53	0.79**	0.87***	0.41
Root C	0.23	0.38	0.81**	0.59	0.47	0.85**	0.79**	0.57
Root N	0.55	0.41	0.84**	0.54	0.60*	0.76**	0.93***	0.5

Changes in C_{mic} / C_{org} Ratio Following Woody Plant Invasion of Grassland

The C_{mic}/C_{org} ratio of grassland soils in the surface 0-15 cm of the profile was approximately 6 % and was 1.5 times greater than C_{mic}/C_{org} ratios of wooded landscape elements (4 %). In contrast, cluster and grove soils at 15-30 cm depth had higher C_{mic}/C_{org} ratios than all other landscape elements across both depths. C_{mic}/C_{org} of drainage woodlands at 15-30 cm was not significantly different from woodland values in the upper 15 cm of the profile. The C_{mic}/C_{org} ratio of grasslands at depth (15-30 cm) was about 2%, which was significantly lower than the grassland value of 6% at 0-15 cm (Fig. 11).

With increasing woody plant stand age, the C_{mic}/C_{org} ratios of grove and drainage woodland soils (0-15 cm) decreased linearly from 6 % in woody plant stands < 30 years old to less than 4% in wooded landscape elements > 60 years. There was no relationship between woody plant stand age and C_{mic}/C_{org} ratios of cluster soils at 0-15 cm or any woodland soil at 15-30 cm depth (Fig. 12).

Changes in qCO_2 Following Woody Plant Invasion of Grassland

The metabolic quotient (qCO_2) was up to 125% higher in wooded landscape elements relative to remnant grassland in the upper 15 cm of the profile (Fig. 11). At depth, the trend was reversed and woodland qCO_2 values were significantly lower (200%) than grassland values. Values for clusters, groves, and drainage woodlands were similar within each depth increment (Fig. 11).

With time following woody plant invasion of grassland, qCO_2 increased linearly by 200-275 % in the upper 15 cm of the profile from 0.4 mg CO_2-C g SMB-C^{-1} hr^{-1} in

grasslands to 1.2 to 1.5 mg CO_2-C g SMB-C^{-1} hr^{-1} in older cluster, grove, and drainage woodlands. At 15-30 cm, there was no relationship between qCO_2 and time following woody plant invasion of grassland (Fig. 12).

DISCUSSION

Woody plant proliferation in grass-dominated ecosystems is prevalent worldwide. However, current understanding of the impacts of woody plant species on grassland biogeochemistry is limited. Discrepancies exist concerning whether or not grassland ecosystems experiencing woody plant encroachment are net C sources or sinks. A recent study by Jackson et al. (2002) predicts decreases in SOC and soil N with increasing mean annual precipitation > 600 mm. In contrast to the results of Jackson et al. (2002), some studies show no changes in SOC or soil N (McCarron et al., 2003; Smith and Johnson, 2003) following woody plant invasion of grasslands, whereas most studies have shown that woody plant establishment in grass-dominated ecosystems results in greater concentrations of SOC and STN in soils beneath tree/shrub canopies (Tiedemann and Klemmedson, 1973; Virginia and Jarrell et al., 1983; Mordelet et al., 1993; Stock et al., 1995; San Jose et al., 1998; Geesing et al., 2000; Burrows et al., 2002; Reyes-Reyes et al., 2002; Yelenik et al., 2004). It is not yet clear why responses to woody encroachment can range from net losses to net gains in soil C and N.

The results of this study, together with those from previous studies at this site (Archer et al., 2001, 2004; Hibbard et al., 2001, 2003; McCulley et al., 2004), indicate that woody plant invasion has altered soil chemical and physical properties and resulted

in substantial increases in SOC and STN beneath woody plant canopies over the past century. These increases are likely a consequence of higher rates of above- and belowground productivity in woodlands relative to adjacent remnant grasslands (Belsky et al., 1993; Mordelet et al., 1993; Boutton et al., 1998; Archer et al., 2001; Hibbard et al., 2001). Additionally, many of the woody species that invade grasslands are capable of symbiotic N-fixation (Virginia and Jarrell, 1983; Johnson and Mayeux, 1990; Stock et al., 1995; Zitzer et al., 1996); the addition of fixed-N to N-limited dryland ecosystems undoubtedly stimulates primary productivity in these systems.

Increases of up to 250% for SOC and STN in wooded landscape elements relative to remnant grassland in this study (Table 5) are almost certainly a direct consequence of higher rates of net primary productivity (NPP) and N-fixation in wooded landscape elements relative to remnant grasslands. Rates of aboveground NPP in remnant grasslands are approximately 1.9-3.4 Mg ha $^{-1}$ yr $^{-1}$; in contrast, rates of aboveground NPP in areas currently dominated by woody vegetation are approximately 5.1-6.0 Mg ha $^{-1}$ yr $^{-1}$ (Archer et al., 2001; Hibbard et al., 2001). Although rates of belowground NPP have not been quantified, coarse and fine root biomass are 2-5 times greater and show significantly larger seasonal fluctuations in wooded areas than remnant grasslands (Boutton et al., 1998, 1999; Hibbard et al., 2001), suggesting that belowground productivity is also significantly accelerated following woody plant establishment in grasslands. Finally, Zitzer et al. (1996) showed that 6 dominant tree legumes from the wooded landscape elements in this study area produce active root nodules with nitrogenase activity, but did not compute ecosystem-level rates of N-

fixation. However, rates of N-fixation in other comparable mesquite woodlands in the southwestern USA have been estimated to range from 4-15 g N m^{-2} yr^{-1} (Rundel et al., 1982; Johnson and Mayeux, 1990). Thus, increases in above- and belowground primary production and rates of N-fixation are potentially able to account for observed increases in SOC and STN in wooded portions of the landscape relative to remnant grasslands.

As hypothesized, these accumulations of SOC and STN in wooded landscape elements resulted in greater SMB-C beneath woody plant canopies (Fig. 11). This trend towards higher SMB-C (0-15 cm) in wooded areas relative to grassland is consistent with previous studies in many other grassland, savanna, and shrubland ecosystems worldwide (Belsky et al., 1989; Kirchmann and Eklund, 1994; Kieft et al., 1998; Reyes-Reyes et al., 2002). Increases in SMB-C were attributed not only to the higher concentrations of nutrients beneath wooded plant canopies but also to the microclimatic amelioration effects of woody plant species, such as increases in soil moisture due to reduced evaporation and reductions in soil temperature due to increased shading (Belsky et al., 1989; Kirchmann and Eklund, 1994; Kieft et al., 1998; Reyes-Reyes et al., 2002).

In this subtropical savanna ecosystem, the positive correlations between SMB-C and SOC, STN, litter C and N, and root C and N suggest that microbes may be C- and/or N-limited. Regression analyses showed that both SOC and STN in wooded landscape elements accounted for 50 to 90% of the variation in SMB-C (Table 6). Additionally, SMB-C in woodlands was also strongly correlated with litter and root C and N. These results suggest that the increases in above- and belowground productivity may help sustain the observed increases in SMB-C in wooded areas. Plant productivity has been

cited as the most important process influencing organic matter accumulation and soil microbial biomass dynamics (Zak et al., 1990). C- and N-limitations in grasslands prior to woody plant establishment were likely due to substantially lower plant productivity. SMB-C is often highly correlated with SOC content at ecosystem (Insam and Domsch, 1988; Insam et al., 1989) and continental scales (Zak et al., 1994) reflecting the dependence of SMB on plant productivity and organic C inputs. In addition to C limitations, many ecosystems are also N limited (Wardle, 1992). In ecosystems that are N-limited, SMB should also be highly correlated with indices of plant productivity since plant production provides the primary substrate for microbial metabolism (Zak et al., 1990). The highly positive correlation between SMB-C and SOC and STN in this study supports this notion. In addition, N amendments to soils in a semi-arid/arid environment in Texas resulted in increased SMB-C also suggesting that microbes in dryland ecosystems may be strongly limited by N-availability (Zhang and Zak, 1998).

Although organic matter inputs to soil have increased following grassland-to-woodland conversion as a result of the change in plant productivity, little is known regarding the quality of woody plant-derived organic matter inputs. In conjunction with SMB-C, the C_{mic}/C_{org} ratio (ratio of SMB-C to SOC) and the metabolic quotient (qCO_2) are indices of microbial activity and are helpful in assessing the quality of the C that has accrued over the past century following woody plant invasion of grassland as well as possible mechanism(s) of C sequestration in this semi-arid ecosystem. In addition to the probable effects of NPP on increased C storage, I hypothesized that SOC could be accumulating in woodlands because 1) the available C in woodland soils may be of

poorer quality than that in grasslands, and 2) microbial communities in wooded landscape elements may be less efficient at converting available C sources to biomass.

The C_{mic}/C_{org} ratio may reflect changes in the quality of soil organic matter inputs associated with land-use/land cover changes (Sparling, 1992). Thus, alterations in substrate availability as a consequence of a shift in organic matter quality should be evident in changes in the C_{mic}/C_{org} ratio. Although inputs from litter and roots are greater in wooded areas, the quality of the C may be lower due to the higher lignin content and concentrations of plant secondary compounds in wooded tissues relative to herbaceous tissues. The pattern of decreasing C_{mic}/C_{org} ratios with time following woody plant invasion (Fig. 12) suggests a decrease in the relative availability of organic matter inputs with time (i.e. less microbial biomass is supported per unit SOC with time following woody invasion). Greater inputs of soil C in woodlands may be inversely related to C quality. In a study in a southern Boreal forest, Bauhus et al. (1998) found a decrease in substrate quality with increasing stand age, which was reflected in a decline in C_{mic}/C_{org} ratio with increasing woody stand age as was similarly observed in this study (Fig. 12). Estimates of longer mean residence times from lab and field determinations for woodland SOC relative to grassland SOC also suggest that organic matter quality is lower in woodlands in this subtropical savanna parkland (Boutton et al., 2002; McCulley et al., 2004). Estimates of C pool sizes from long-term soil incubations indicated proportionally smaller labile C pools in woodlands (10-20%) relative to remnant grasslands (35%) suggesting that a larger proportion of woodland-derived C inputs is slow/resistant to decomposition (Boutton et al., 2002). These studies strongly support

the notion that although quantity of organic matter has increased following grassland-to-woodland conversion, the quality of the organic matter has decreased as evidenced by the lower C_{mic}/C_{org} ratios in woodlands relative to grasslands (Fig. 11).

In contrast to decreasing C_{mic}/C_{org} ratios with time following woody plant invasion of grassland, qCO_2 (metabolic quotient) increased with woody plant stand age at 0-15 cm (Fig. 12). The higher qCO_2 in wooded areas reflects microbial inefficiency at converting available C to biomass; that is, more respiration is required to produce a unit of microbial biomass in wooded areas. The reduced efficiency of soil microbes in converting available C to biomass in wooded areas may be due to the lower quality of the woodland-derived organic matter inputs, as also suggested by lower C_{mic}/C_{org} ratios in wooded areas. Microbial efficiency of C utilization of lignin for microbial biomass production is low with most of the carbon being evolved as CO_2 (reflected by a higher qCO_2) or incorporated into soil organic matter (Wagner and Wolf, 1998). Higher qCO_2 in woodlands relative to grassland may also be indicative of a shift in microbial composition. Because fungi and bacteria differ in their abilities to convert available C to biomass, shifts in fungal: bacterial ratios may be reflected in changes in qCO_2. Fungi are considered to be more efficient at converting available C to biomass than bacteria (Sakamoto and Oba, 1994). With increasing woody inputs that are higher in lignin than herbaceous tissue, it might be expected that the fungi: bacteria ratio would increase resulting in lower qCO_2 in wooded areas. However, a recent study showed that the fungi: bacteria ratio was actually lower in soils beneath tree legumes relative to open areas (Reyes-Reyes et al., 2002). This suggests that qCO_2 may increase following

grassland-to-woodland succession if bacterial populations increase relative to fungal populations in soils beneath wooded plant canopies. The ratio of fungi to bacteria was not evaluated in this study so no assumptions may be made in this regard although higher qCO_2 in woodlands may be consistent with a lower fungi:bacteria ratio in woodland soils. Although a variety of factors may affect C_{mic}/C_{org} and qCO2, evaluation of the two indices together (C_{mic}/C_{org} and qCO_2) indicates that the most apparent explanation for lower C_{mic}/C_{org} and higher qCO_2 in woodlands appears to be that organic matter quality has decreased following grassland to woodland conversion.

Changes in the size and activity of SMB have implications at both ecosystem and global scales. Previous field studies at this study site have shown that soil respiration and net N-mineralization are greater in wooded areas than in remnant grasslands (Hibbard et al., 2001; McCulley et al., 2004). Thus, the larger pool sizes of SMB observed in this study and in McCulley et al. (2004) are accompanied by higher rates of key processes in the C and N cycles. More rapid rates of C and N cycling may lead to enhanced soil nutrient availability, thereby altering plant-microbe and plant-plant interactions, and potentially influencing competitive interactions and successional dynamics in this ecosystem.

Changes in soil structure are also influenced by changes in the size and activity of SMB. Another recent study at this site suggests that woody plant encroachment of grasslands has resulted in detectable changes in soil structure (See Chapter 5). It appears that more stable aggregates are being formed following the vegetation shift from grassland to woodland. The increased formation of stable aggregates following woody

plant encroachment is likely facilitated in part by the greater SMB in wooded areas compared to remnant grasslands. SMB has shown positive correlations with aggregate size and stability (Gupta and Germida, 1988; Drury et al., 1991), and the residues of SMB are critical in the formation and stabilization of soil aggregates (Tisdall and Oades, 1982; Oades and Waters, 1991; Jastrow and Miller, 1998) which play an important role in soil structure. Soil structure in turn is very important in the protection and storage of soil organic matter (Christensen, 2001; Six et al., 2002a). Soil structure also influences other soil functions and hydrologic processes such as compaction, infiltration, erosion, runoff, and water-holding capacity (Carter, 2002).

The larger pools of SMB in wooded areas also result in higher rates of trace gas fluxes such as CO_2 (McCulley et al., 2004) and NO_x (Cole et al., 1996). CO_2 is an important greenhouse gas, and NO_x is an important reactant in the formation of tropospheric O_3. Thus, the larger SMB-C pools in wooded areas imply that woody encroachment into grasslands is an important ecological process that could influence atmospheric chemistry and the climate system.

CONCLUSIONS

Size of the SMB increased following woody plant invasion of grassland. The increases in SMB-C are likely due to the greater above- and belowground productivity of woodlands relative to remnant grassland resulting in greater organic matter inputs. SOC and STN increased following woody plant invasion of grasslands and were positively correlated with SMB-C indicating that microbes may have been nutrient-limited prior to

woody plant proliferation in this dryland ecosystem. Although absolute values of SMB-C increased following woody plant invasion of grassland, the concomitant linear decreases in the C_{mic}/C_{org} ratio with time indicates that the quality of the C available to microbes may have decreased over time as less microbial biomass is supported per unit organic C. Increases in qCO_2 with time following woody invasion also suggest that organic matter quality may be reduced since microbes appear to be less efficient at converting available C to biomass. Increases on SOC and STN may be a function of both greater inputs of poor quality C that is relatively resistant to decay and the decreased ability of soil microbes to decompose this organic matter. Thus, both the size and activity of SMB-C appear to be useful indices in evaluating the biogeochemical impacts of vegetation change and associated changes in organic matter quality. Additionally, alterations in the size and activity of the SMB have the potential to influence ecosystem and global level functions and processes related to the C and N cycles.

CHAPTER V

STORAGE AND DYNAMICS OF CARBON AND NITROGEN IN SOIL PHYSICAL FRACTIONS FOLLOWING WOODY PLANT INVASION OF GRASSLAND

INTRODUCTION

Woody plant encroachment is prevalent in many grass-dominated ecosystems worldwide. This globally extensive phenomenon has been documented in North and South American, Africa, Asia, and Australia and appears to be driven primarily by human land-use practices, especially livestock grazing and fire suppression (Archer et al., 2001; Van Auken, 2000). Grass-dominated ecosystems cover 50% of the terrestrial surface and store >30% of global soil organic C (SOC) (Schlesinger, 1997). Therefore, this widespread shift in ecosystem structure has strong potential to alter key ecosystem processes that could affect C and N cycles at ecosystem, regional, and global scales. In fact, it has been estimated that woody encroachment into grasslands may represent as much as 34% of the ecosystem C sink strength in the USA alone (Houghton et al., 1999; Tilman et al., 2000; Pacala et al., 2001).

As a case in point, trees and shrubs have invaded areas previously dominated by grasses in the subtropical Rio Grande Plains of southern Texas during the past 150 yrs (Archer et al., 1988, 2001; Boutton et al., 1998, 1999). Prior studies in this region have indicated that the invasive woodlands are dominated by N-fixing trees/shrubs (Zitzer et al., 1996), have higher rates of net primary productivity than the grasslands they replace (Archer et al., 2001, 2004; Hibbard et al., 2001, 2003), and these higher rates of

productivity and N-input have resulted in increased C and N storage in biomass (Archer et al., 2001) and soils (Boutton et al., 1998; Archer et al., 2004; McCulley et al., 2004) in wooded portions of the landscape. However, relatively little is known regarding the mechanisms by which these elevated C and N inputs are stabilized and stored in the soil environment. This shift in vegetation structure from grasslands to woodlands dominated by N-fixing trees/shrubs provides a unique opportunity to look at the effects of changes in ecosystem structure (i.e. grassland to woodland) and function (increased productivity and N-inputs) on SOC and soil total N storage and dynamics over the last 100-130 years.

Recently, soil physical structure has been emphasized as a determinant of soil organic matter sequestration and turnover (Elliott, 1986; Jastrow and Miller, 1998; Six et al., 1998; Ladd et al., 1993). Within the soil structure context, soil organic matter occurs in different sizes, shapes, and densities, and occupies different positions within the soil matrix which results in differential accessibility of the soil organic matter to soil organisms; this results in organic matter pools that differ in stability and dynamics (Ladd et al., 1993). The conceptual view of aggregate hierarchy suggests that the process by which soil organic matter is incorporated into the soil matrix occurs with some degree of order (Tisdall and Oades, 1982; Oades, 1993). In areas where biotic factors are the main agents of aggregation, soil primary particles (sand, silt, clay) are stabilized into secondary and higher orders of aggregates. Primary particles are cemented together by clay-organic matter complexes incorporating highly decomposed aromatic humic aliphatic substances and polysaccharides. Microaggregates often have cores of partially decomposed particulate organic matter (POM) and are considered stable against

disturbance (Oades and Waters 1991). Organic matter associated with this fraction is characterized as older and more resistant to decay (Six and Jastrow, 2002). Microaggregates and other primary particles are bound into macroaggregates by roots and fungal hyphae and by polysaccharides (more labile, easily decomposable forms of C). Because of the relatively labile, ephemeral nature of the binding agents and the size of pores being bridged by binding agents, macroaggregates are considered less stable than microaggregates (Tisdall and Oades, 1982).

Thus, soil organic matter is comprised of fractions differing in physical and chemical properties, decomposability, and turnover. Physical fractionation procedures utilize differences in the size and density of organic matter pools that differ in structure and function and, hence, storage mechanisms and turnover. Although physical fractionation of SOC has been widely employed in the study of land cover/land use effects in agricultural systems, it has seldom been applied to the study of SOC and soil total N storage and dynamics in natural systems that have never been cropped or cultivated. The specific objectives of this study were to elucidate the role of soil structure in C and N sequestration following woody invasion of grassland and to quantify the dynamics and longevity of C and N in organic matter fractions isolated from specific physical locations within the soil structure.

MATERIALS AND METHODS

Study Area

Field sampling was conducted in December 2001 at the Texas Agricultural Experiment Station LaCopita Research Area (27° 40'N, 98° 12'W) located 65 km west of Corpus Christi, Texas, in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical with mean annual temperature of 22.4°C and mean annual precipitation of 716 mm (peaks in May-June and September). The topography consists of nearly level uplands that grade (1-3% slopes) into lower-lying drainage woodlands. Elevation ranges from 75-90 m. Evidence from sequential aerial photography, tree ring analyses, vegetation dynamics modeling, and the isotopic composition of soils all indicate that this region was once relatively open grassland and that woody plant encroachment into grasslands has occurred over the past 150 years (Archer et al., 1988, 2001, 2004; Boutton et al., 1998, 1999). Domestic livestock have grazed this research area over the past century, but soil samples were collected from a grazing exclosure that was established in 1985.

Upland surface soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic (B_t) horizon that includes non-argillic inclusions. Uplands are dominated by C_4 grasslands interspersed with small, discrete clusters of woody plants. Dominant C_4 grasses in uplands include species of *Paspalum*, *Bouteloua*, Chloris, and *Eragrostis*. Clusters expand laterally, fusing to form larger groves of woody vegetation where the argillic horizon is absent. In lower-lying portions of the

landscape, soils are finer-textured clay loams (Pachic Argiustolls) and support closed-canopy drainage woodlands.

Prosopis glandulosa [Torr.] var. *glandulosa* (honey mesquite) is the dominant plant species in all wooded landscape elements. Mesquite is the first to colonize in grasslands and subsequently facilitates recruitment of other woody plant species beneath its canopy. Understory species common to both uplands and lowlands include *Condalia hookeri* (M.C. Johnst.), *Celtis pallida* (Torr.), *Zanthoxylum fagara* (L.), *Diospyros texana* (Scheele.), *Zizyphus obtusifolia* (T.&G.), and *Beberis trifoliolata* (Moric.). Plant nomenclature follows Correll and Johnston (1979). Additional details on plant communities and soils have been presented elsewhere (Scifres and Koerth, 1987; Archer et al., 1988; Boutton et al., 1998).

Chronosequence Approach

A chronosequence approach was used to quantify C and N pool sizes and their rates of change in soils and soil fractions following woody plant encroachment into areas that were previously open grasslands. Ten sites were sampled within remnant grasslands, clusters, and groves (upland sites); eleven sites were sampled within lower-lying drainage woodlands. All sites were located within an area of approximately 2 km^2. Remnant grasslands were sampled to characterize C and N pool sizes at time zero (i.e. prior to woody encroachment), whereas clusters, groves, and drainage woodlands of known age were sampled to evaluate changes in C and N pools at different points in time following woody invasion.

Ages of clusters, groves, and drainage woodlands were based on the fact that the formation of these wooded landscape elements is initiated only after the establishment of *Prosopis glandulosa* in grasslands (Archer et al., 1988). Thus, the age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand. The ages of *P. glandulosa* trees were determined by measuring their basal diameters, and then using those values to predict tree ages based on regression equations specific to each landscape element (Stoker, 1997). Woody plant stands sampled in this study were selected to encompass the full range of *P. glandulosa* basal diameters, corresponding to tree ages ranging from approximately 10-130 years.

Collection of Soil Samples

Four soil cores (5-cm diameter x 30-cm length) were taken, one in each cardinal direction within 0.5 m of the bole of the largest *P. glandulosa* at each wooded site. Sampling was identical in each remnant grassland site, but cores were taken in each cardinal direction from the base of a perennial C_4 grass plant. Soil surface litter was removed gently to expose mineral soil prior to taking each soil core. All soil cores were sectioned into 0-15 and 15-30 cm increments and stored at 4^{o}C. In the lab, each soil sample was mixed thoroughly, and a subsample was dried at 105^{o}C to determine bulk density. Then, the 4 cores from each site were pooled by depth increment and mixed for a composite sample.

Soil Chemical and Physical Characterization

An aliquot of soil was passed through a 2-mm sieve to remove large organic matter fragments and used for physical, chemical, and elemental analyses. Soil pH was

determined on a 1:2 (soil:water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipette method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was dried at 60^{o}C, pulverized in a centrifugal mill, and used for elemental analyses.

Soil Physical Fractionation

An aliquot of field-moist soils was first passed thru a 15-mm sieve followed by an 8-mm sieve to remove large roots and then thoroughly mixed and air-dried. Soils were physically fractionated (Fig. 13) into 2 size classes of water-stable aggregates by using the disruptive forces of slaking and wet-sieving, and aggregates were further dispersed into POM and mineral components by shaking in sodium hexametaphosphate (HMP) by following a procedure modified from Elliott (1986) and Six et al. (1998). After oven-drying overnight at 65^{o}C, a subsample of soil from each site was immersed in deionized water on top of a 250-μm sieve. Organic debris and root pieces that floated in the water (density <1.0 g cm^{-3}) were aspirated onto a 20-μm nylon filter. This material was quantified as the free light fraction (Free LF) and considered to be the litter component of whole-soil C.

After 5 minutes of slaking, the sieve was manually moved up and down 3 cm, 50 times over a 2 minute period. The fraction remaining on the 250-μm sieve was oven-dried at 65^{o}C and collected in a pre-weighed aluminum pan. Water plus soil < 250 μm was poured through a 53-μm sieve and the sieving procedure was repeated. Unaggregated (free) silt and clay that passed through the 53-μm sieve was collected by centrifugation. This procedure yielded a macroaggregate-sized fraction >250 μm

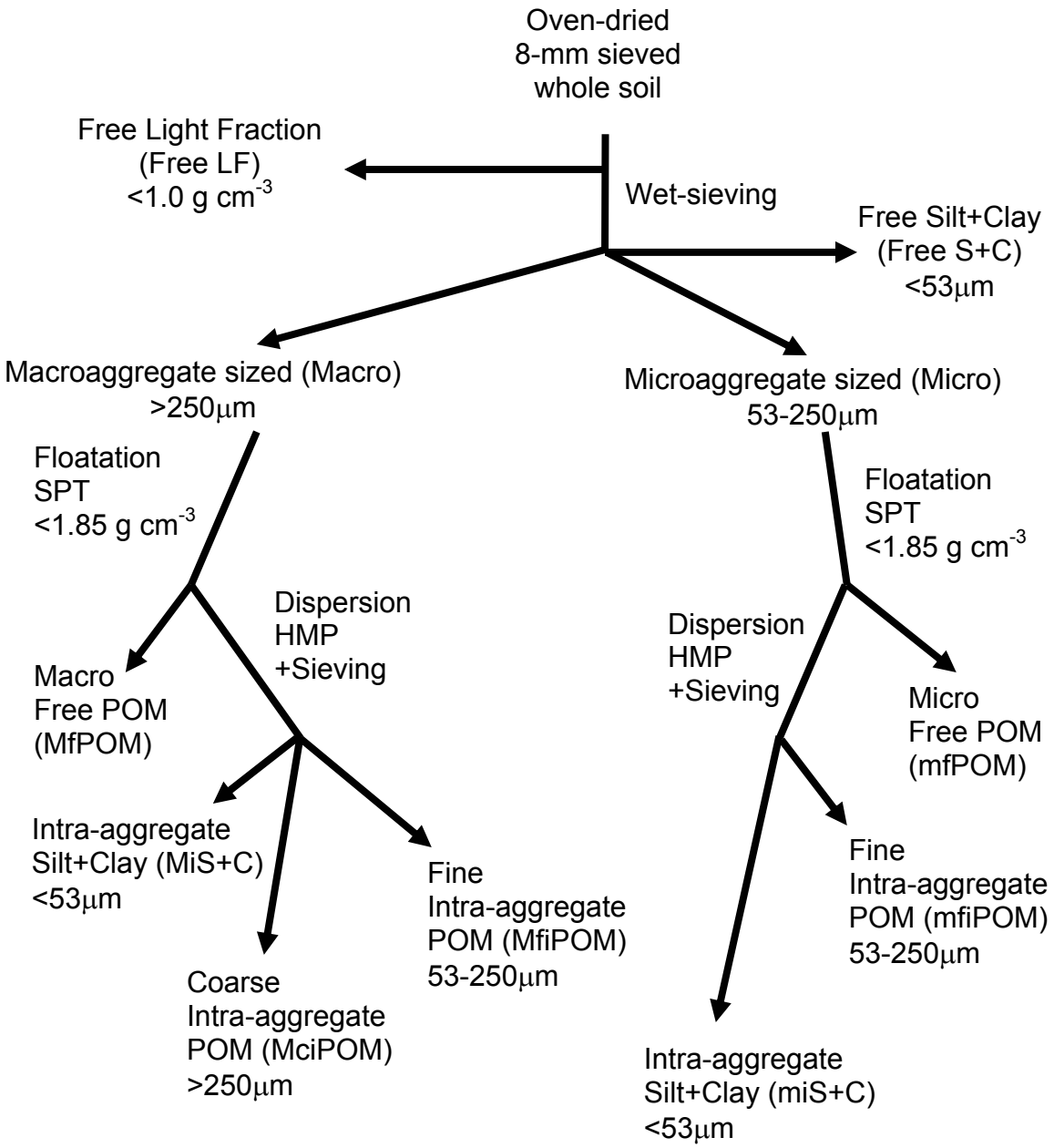

Fig. 13. Flow chart for separation of soil organic matter into different size and density fractions (adapted from Elliott, 1986 and Six et al., 1998). POM=particulate organic matter, SPT=sodium polytungstate, HMP=sodium hexametaphosphate.

(Macro), a microaggregate-sized fraction 53-250 μm (Micro), and a free silt+clay fraction <53 μm (Free S+C). Silt and clay were not quantified separately because of the small percentage of silt and clay relative to whole soil at all sites (Table 7).

Both aggregate-sized fractions consisted of intact aggregates, sand of the same size class, and inter-aggregate POM of the same size class released during slaking and sieving. Inter-aggregate POM was separated from intact aggregates and sand by flotation of 5-g subsamples in sodium polytungstate (SPT) at a density of 1.85 g cm^{-3}. This material (MfPOM and mfPOM) was aspirated onto a 20-μm nylon filter, and washed with deionized water and dried. The remaining heavy fractions were washed with deionized water 3 times to thoroughly remove SPT. The heavy fractions were dispersed by shaking for 16 hours on a reciprocating shaker at low speed (180 oscillations per minute) in 5 g L^{-1} of HMP solution. Dispersed macroaggregates were passed sequentially through 250 and 53-μm sieves and rinsed with deionized water to yield coarse intra-aggregate POM >250 μm (MciPOM), fine intra-aggregate POM 53-250 μm (MfiPOM), and macroaggregated silt+clay (MiS+C). Dispersed microaggregates were passed through a 53- μm sieve to yield fine intra-aggregate POM 53-250 μm (mfiPOM) and microaggregated silt+clay (miS+C). Intra-aggregate silt+clay <53 μm (iS+C) was collected after addition of 5.0 ml of 0.25M $CaCl_2$ + 0.25M $MgCl_2$ to flocculate clays.

Table 7. Soil physical and chemical characteristics of landscape elements (grassland, cluster, grove, drainage) at LaCopita Research Area. Different letters represent significant differences between means within a row for each depth interval from Fisher's LSD following ANOVA. Standard errors of the mean are in parentheses.

	0-15 cm				15-30 cm			
	Grassland	**Cluster**	**Grove**	**Drainage**	**Grassland**	**Cluster**	**Grove**	**Drainage**
pH	6.5 (0.1)^{a}	6.4 (0.06)^{a}	6.3 (0.1)^{a}	6.2 (0.1)^{a}	6.9 (0.06)^{a}	6.4 (0.06)^{b}	6.7(0.05)^{a}	6.3 (0.1)^{b}
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Clay Loam
Sand (%)	80.5 (0.40)^{a}	81.3 (0.5)^{a}	81.4 (0.6)^{a}	66.0 (3.0)^{b}	74.1 (1.5)^{a}	78.8 (0.9)^{a}	78.0 (0.8)^{a}	56.1 (4.1)^{b}
Silt (%)	10.5 (2.0)^{ab}	9.6 (1.6)^{b}	12.0 (1.3)^{ab}	15.4 (2.7)^{a}	16.9 (1.9)^{ab}	12.5 (2.2)^{ab}	8.9 (2.2)^{c}	21.0 (2.1)^{a}
Clay (%)	9.0 (2.0)^{b}	9.1 (1.6)^{b}	6.6 (1.3)b	18.6 (1.9)^{a}	9.1 (2.3)^{b}	8.7 (2.0)^{b}	13.1 (1.8)^{b}	22.9 (2.8)^{a}
Bulk Density (g cm^{3})	1.2 (0.02)^{a}	1.0 (0.03)^{b}	1.0 (0.02)^{b}	1.0 (0.04)^{b}	1.3 (0.03)^{a}	1.3 (0.02)^{ab}	1.2 (0.02)^{ab}	1.2 (0.02)^{b}

Recovery of Soil Mass and C and N Concentrations Following Fractionation

Approximately 99.5% of whole-soil mass was recovered in soil fractions isolated by wet-sieving. Following dispersion, 94.1% of macro- and microaggregate mass was recovered in soil fractions. Lower recovery was likely related to loss of sand close to the size of sieve openings during collection and washing of fractions from sieves. Recovery of C and N in fractions from wet-sieving was 98% and 92% of whole-soil C and N respectively. However, recovery of C and N in fractions following dispersion indicated 25-30% of whole-soil C and N was lost. C and N may have been lost following flotation of macro- and microaggregate-sized fractions in SPT to remove the free POM fraction and/or following dispersion of the remaining heavy fraction in sodium hexametaphosphate. Chan (2001) found that as much as 20% of total organic C was lost and recovered as soluble organic C in the sodium hexametaphosphate solution following dispersion of soil.

Elemental Analyses

Soil fractions isolated by wet-sieving and dispersion were dried at 65^{o}C in a convection oven, weighed, and ground in a SPEX mill (SPEX CertiPrep, Metuchen, NJ). Whole-soil and soil fractions were analyzed for C and N concentrations on a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ). None of the samples contained $CaCO_3$. Concentrations of C in Macro and Micro free POM were subtracted from macro- and microaggregate-sized fractions so that reported concentrations for these fractions only reflect organic matter contained within intact water-stable aggregates. Macroaggregate-sized fractions contained little to no sand >250 μm. Because microaggregate-sized

fractions contained sand of the same size class as the microaggregates, all concentrations are reported on a whole-soil basis (g C or N in fraction kg^{-1} soil).

Statistical Analyses

ANOVA was used to determine differences between soil physical and chemical characteristics of grassland and woodland landscape elements (0-15 and 15-30 cm) at LaCopita Research Area in southern Texas. Fisher's LSD method was used to distinguish differences among landscape elements within a soil depth following ANOVA. Linear regression analyses were used to determine significant relationships between size distributions of aggregate mass and increasing woody plant stand age (i.e. time following woody plant invasion). Linear regression analyses were also used to determine significant relationships between C and N concentrations and woody plant stand age. C and N concentrations were converted to C and N stocks by using soil bulk density estimates for each soil sample at each site. The slopes of regression lines fit to C and N stocks were used to determine soil C and N accumulation rates (g C or N m^{-2} yr^{-1}). Because the mass distribution of soil fractions at 15-30 cm were unaffected by woody plant encroachment, regression analyses were only performed for fractions from the upper 15 cm of the profile. ANOVA was used to determine differences among landscape elements for C:N ratios. However, because no significant differences were detected, C:N ratios were averaged across landscape elements to obtain a mean value for each soil fraction. ANOVA was also used to determine differences among landscape elements for concentrations of protected C (POM within macro- and microaggregates and associated with silt+clay) and unprotected C (POM external to aggregates including

MfPOM, mfPOM, and the Free LF) and proportion of protected C. Mean separations were performed using Fisher's LSD. Linear regression analyses were performed using SigmaPlot 2001 (SPSS Inc., 2001). ANOVA were run using Number Cruncher Statistical Systems 6.0 (NCSS, 1995). Significance level is $p<0.05$.

RESULTS

Soil Physical and Chemical Characteristics

Soil pH was approximately 6.5 in the upper 15 cm of the profile in remnant grasslands and was not altered following woody plant encroachment into grasslands (Table 7). Upland soils beneath grasslands, clusters, and groves were loamy sands, with a particle size distribution of approximately 80% sand, 10% silt, and 10% clay. In contrast, soils in lower-lying drainage woodlands were sandy loams with a particle size distribution of 60% sand, 20% silt, and 20% clay (Table 7). Patterns were similar at 15-30 cm, although all soils at this depth contained slightly more silt and clay relative to 0-15 cm. Bulk density of the 0-15 cm depth interval decreased significantly from 1.2 in grasslands to 1.0 in wooded landscape elements (clusters, groves, and drainage woodlands); at 15-30 cm, bulk densities ranged from 1.2-1.3.

Distribution of Soil Fractions by Mass

The distribution of soil fractions (from wet-sieving) as a proportion of whole-soil mass increased in the order Free LF < Free S+C < Macro < Micro (Fig. 14). With time following woody plant invasion of grasslands, relative proportions of the free light fraction and macroaggregate-sized fraction increased linearly in the upper 15 cm of the

soil. The free light fraction (density <1.0 g cm^{-3}) increased from 1% of whole-soil weight in grasslands to 2-4% of whole-soil weight in wooded landscape elements. The macroaggregate-sized fraction (>250 μm) in the 0-15 cm depth increment increased from <10% of whole-soil weight in grasslands to >30% of whole-soil weight in older (>80 yrs) clusters, groves, and drainage woodlands. Consequently, the relative proportions of the microaggregate-sized fraction (53-250 μm) and free silt+clay fraction (<53 μm) decreased linearly with time after woody establishment. Microaggregates decreased from 80% of whole-soil weight in grasslands to approximately 60% of whole-soil weight after 80-130 years of woodland development. The free silt+clay fraction decreased from 11% of whole-soil weight in grasslands to 3-8% in older woodlands (>80 yrs). Unlike the results from wet-sieving, soil fractions as a proportion of total macro- and microaggregate mass showed no significant trends with time following woody plant invasion of grassland. At 15-30 cm, distribution of soil fractions remained largely unaffected by woody plant invasion of grassland.

C and N Concentrations in Whole Soil and Soil Fractions

Total SOC and soil total N (0-15 cm) increased approximately 100-500% along the chronosequence following woody plant invasion of grassland (Fig. 15 and 16). Carbon concentrations of all fractions (0-15 cm) isolated from wet-sieving, except free silt+clay, increased linearly with woodland age (Fig. 15). The greatest increase of C was in organic matter external to aggregates. Carbon concentrations in these non-protected free POM fractions (Free LF + MfPOM + mfPOM) increased up to 1100% from

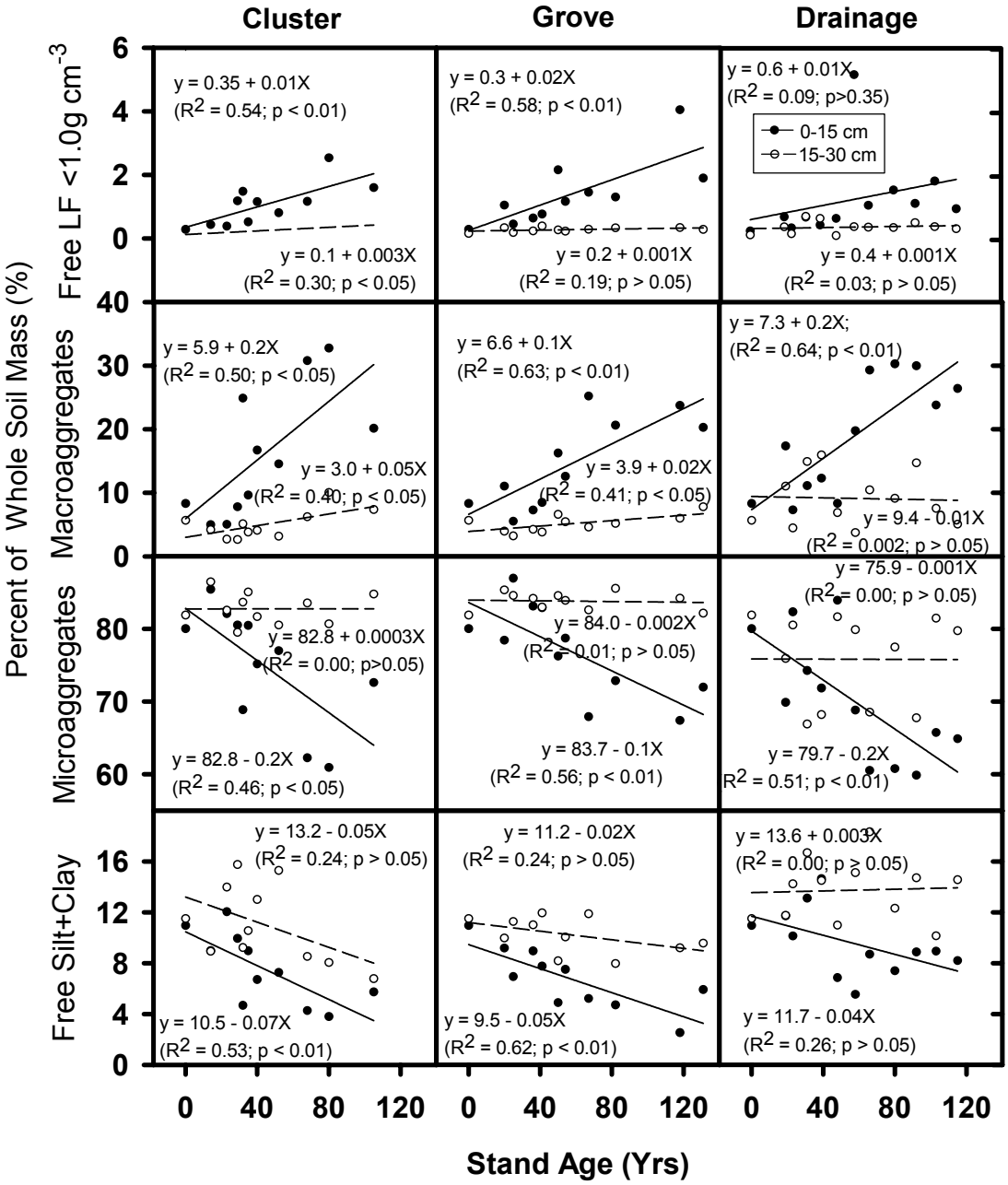

Fig. 14. Soil physical fractions (as a percentage of whole-soil weight) relative to woody plant stand age and soil depth (0-15 and 15-30 cm). Fractions were isolated according to the procedure in Fig. 13. Values for time zero in each frame are remnant grasslands.

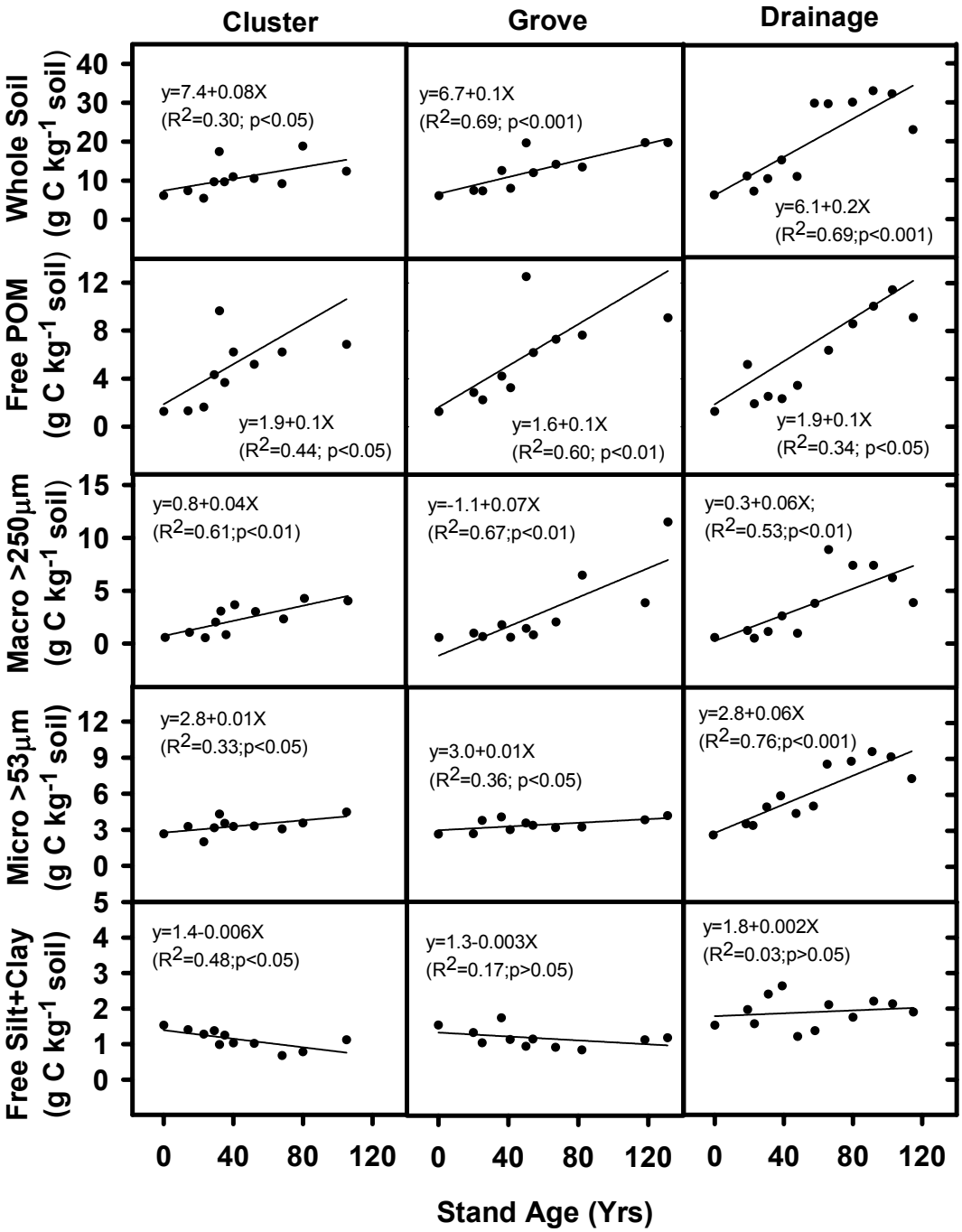

Fig. 15. Carbon (g C kg^{-1} soil) in whole soil and soil fractions relative to woody plant stand age at LaCopita Research Area. In each frame, values for time zero are remnant grasslands.

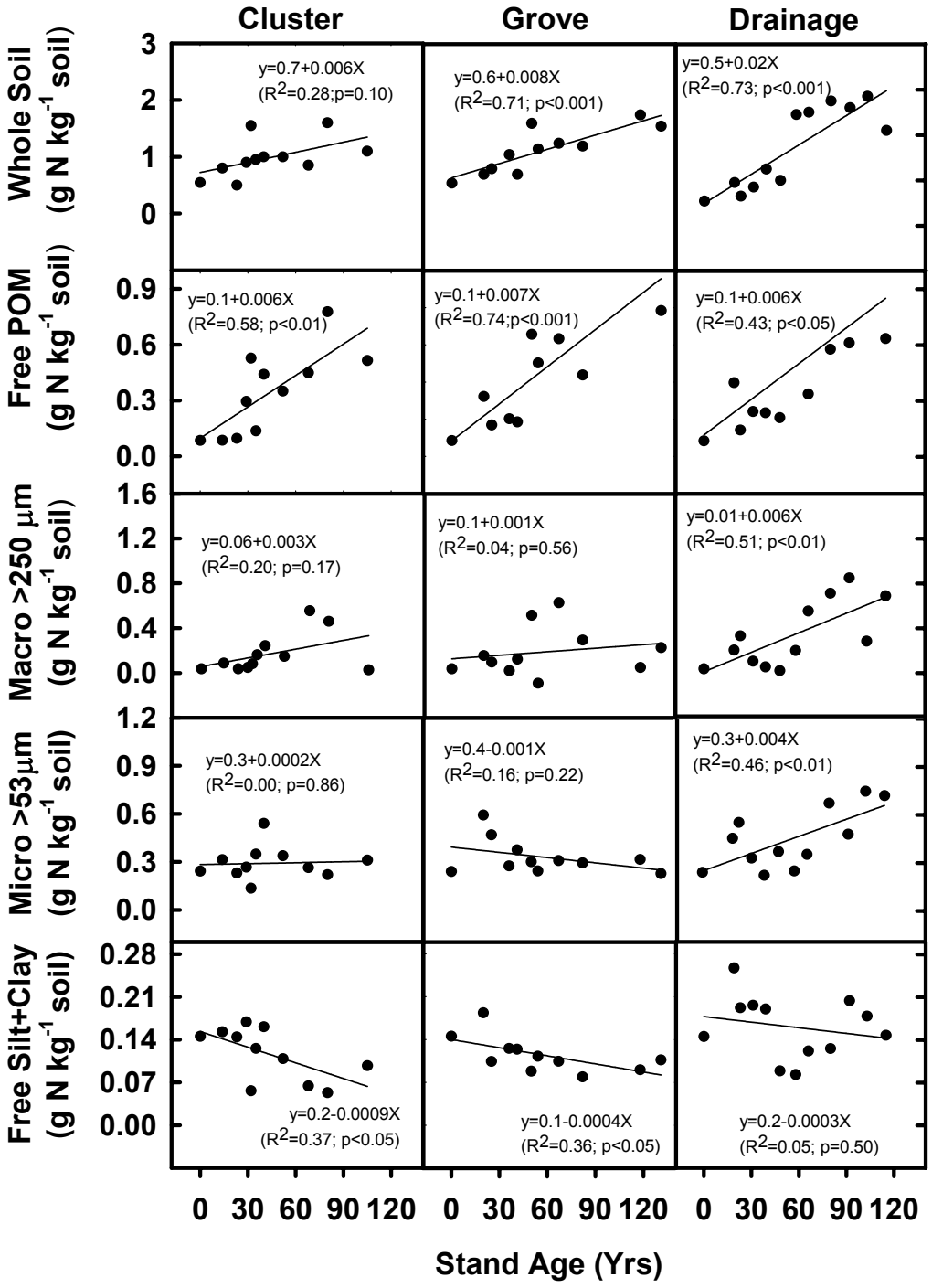

Fig. 16. Nitrogen (g N kg^{-1} soil) in whole soil and soil fractions relative to woody plant stand age at LaCopita Research Area. In each frame, values for time zero are remnant grasslands.

approximately 1 g C kg^{-1} whole soil in grasslands to 12 g C kg^{-1} whole soil in older woodlands. Carbon concentration in macroaggregate-sized fractions increased 300-700% from 1 g C kg^{-1} soil in grasslands to 4-8 g C kg^{-1} soil in older woodlands. Microaggregate C concentrations increased 70-230% from 3 g C kg^{-1} whole soil to 10 g C kg^{-1} whole soil in older drainage woodlands following 130 years of woodland development. C concentrations of the free silt+clay fractions tended to decrease following woody plant invasion although trends were generally not significant (Fig. 15).

Macroaggregate-associated fractions (MciPOM, MfiPOM, MiS+C) in all wooded landscape elements showed significant linear increases in C concentration with time following woody plant invasion of grassland (Fig. 17). All microaggregate-associated fractions (mfiPOM, miS+C) in drainages showed significant increases with time following woody plant invasion of grassland. However, for clusters and groves, although intact microaggregates increased linearly, none of the individual microaggregate-associated fractions showed significant linear increases over time.

Nitrogen concentrations paralleled those of C with the free POM (0-15 cm) showing the largest increases of N with time after woody plant invasion (Fig. 16). Nitrogen concentrations in the free POM fraction (external to aggregates) increased up to 800% from 0.1 g N kg^{-1} whole soil in grasslands to 0.9 g N kg^{-1} whole soil in older groves and drainage woodlands. For both macro- and microaggregate-sized fractions, only drainage woodlands showed significant increases in N concentration of 75 and 130%, respectively, with time after woody invasion (Fig. 16). Nitrogen concentrations of the free silt+clay fraction in clusters and groves showed decreases of 50-88% but

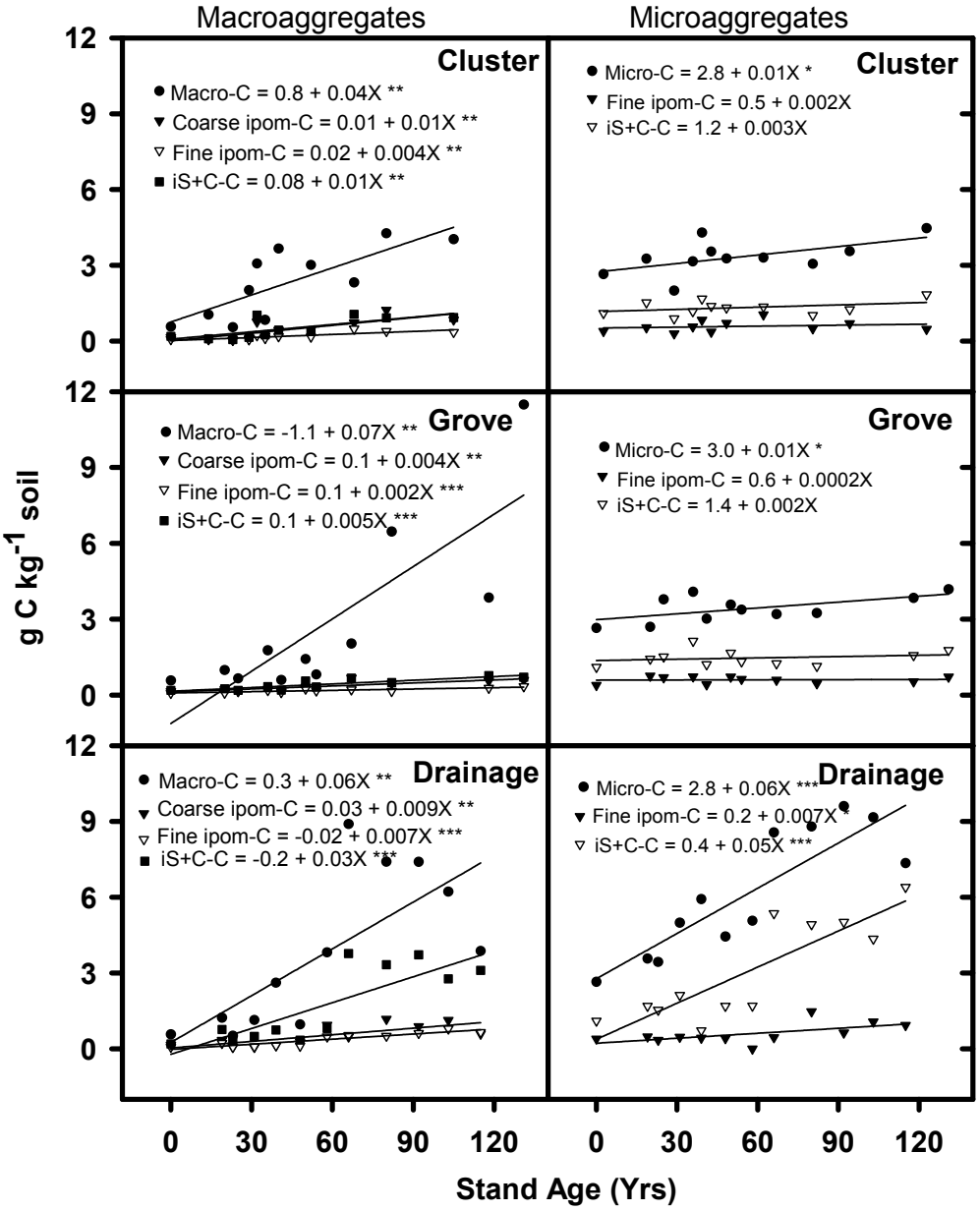

Fig. 17. Carbon (g C kg^{-1} soil) in macro- and microaggregates and aggregate-dispersed fractions relative to woody plant stand age at LaCopita Research Area. Values for time zero in each frame are remnant grasslands.

were not significantly different in drainages following woody plant invasion. Macroaggregate-associated fractions (MciPOM, MfiPOM, MiS+C) showed increases in N concentration with increasing time following woody plant invasion of grassland although the trends were not all significant (Fig. 18). Microaggregate-associated fractions (mfiPOM, miS+C) showed no consistent trends (Fig. 18).

C and N concentrations in unprotected free POM and macroaggregates as a proportion of total whole soil C and N showed increases with time following woody plant invasion of grassland (data not shown). In contrast, C and N as a proportion of total C and N decreased in microaggregates and free silt+clay with time following woodland development. Carbon and N within macroaggregate-associated fractions (MciPOM, MfiPOM, MiS+C) as a proportion of total macroaggregate C and N did not change following woody plant invasion of grassland. Relative to total microaggregate C and N, C and N in microaggregate-associated fractions (mfiPOM, miS+C) also showed no significant proportional changes following woody plant establishment in grasslands.

Unprotected vs. Aggregate-protected C

Relative proportions of protected C (silt+clay and aggregate-associated) were higher in grasslands (80%) than woodlands younger than 50 years old (65-70%) (Fig.19). In woodlands older than 50 years, relative proportions of protected C were lower than those of younger woodlands, ranging from 55-65%. Partitioning by source of protection (data not shown), microaggregates accounted for 50% of the total amount of protected C across landscape elements. In grasslands, most of the protected C was due

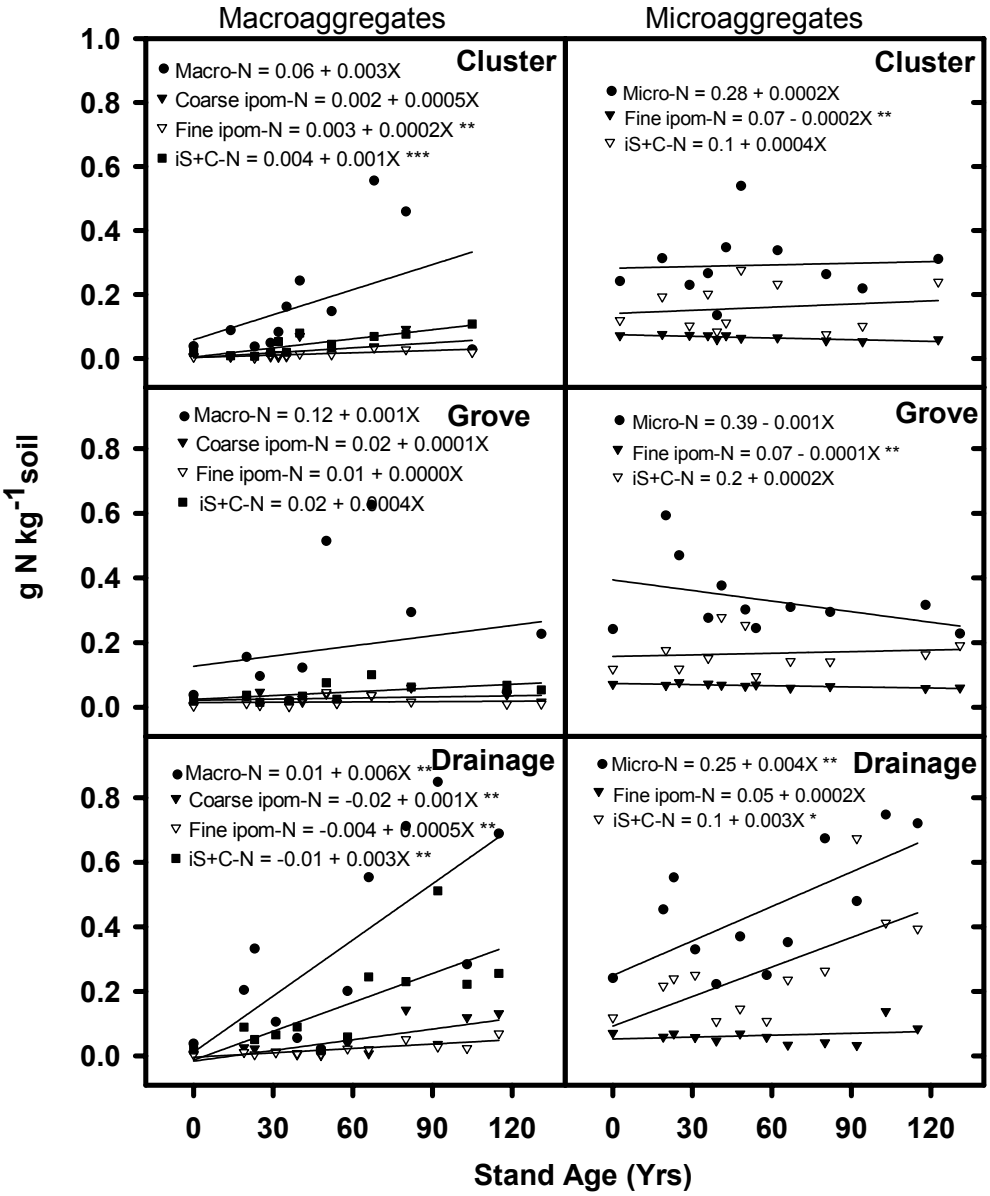

Fig. 18. Nitrogen (g N kg^{-1} soil) in macro- and microaggregates and aggregate-dispersed fractions relative to woody plant stand age at LaCopita Research Area. In each frame, values for time zero are remnant grasslands.

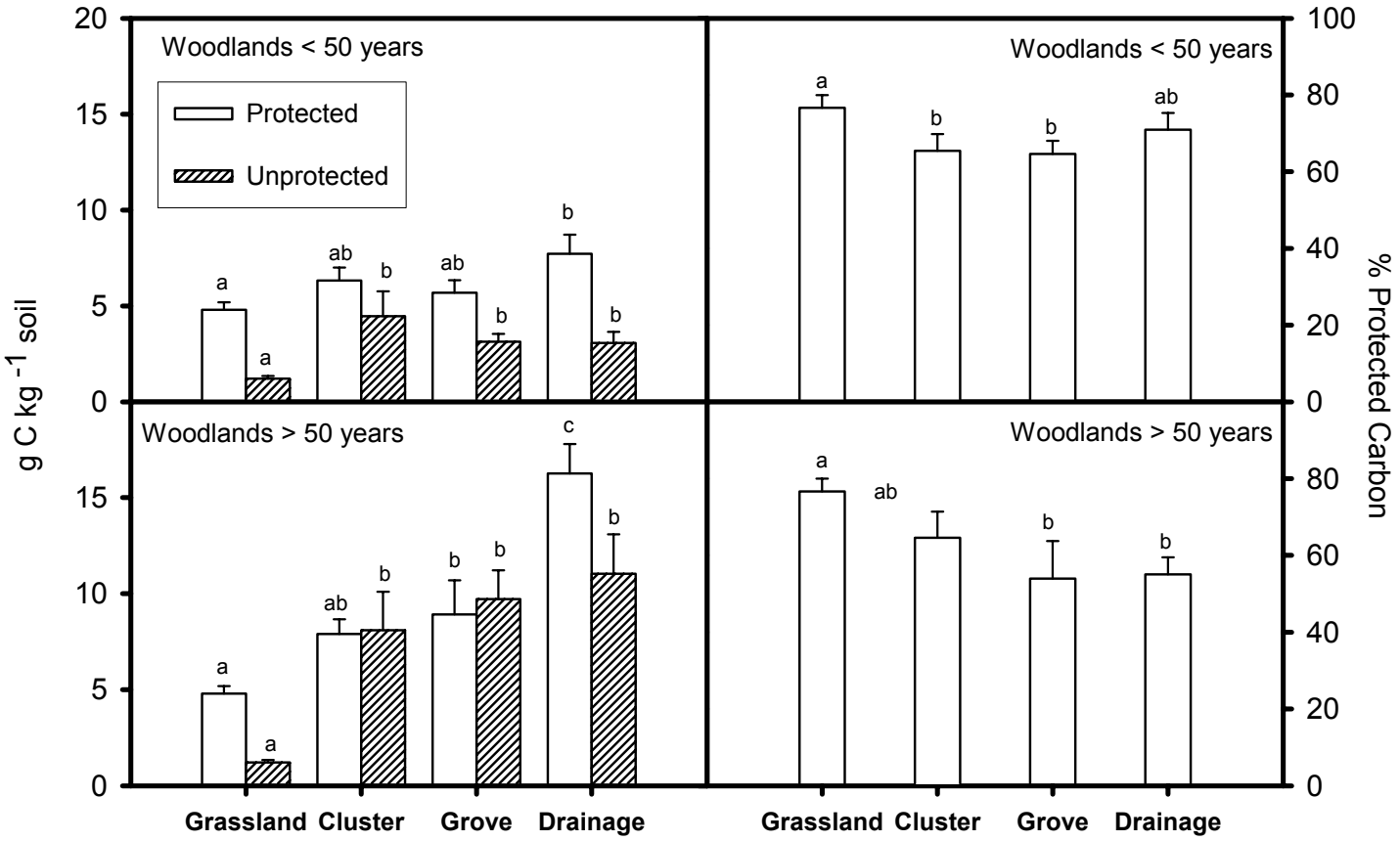

Fig. 19. Protected and unprotected carbon (g C kg^{-1} soil) and proportion of protected carbon in younger (<50 years) and older (>50 years) woodlands relative to remnant grasslands. Different letters above bars within a category (protected vs. unprotected) indicate significant differences among means from Fisher's LSD following ANOVA.

to microaggregates (56%) and silt+clay (32%). In woodlands, protection was due mainly to microaggregates (50%) and macroaggregates (35%). When looking at absolute amounts of protected C, values for grasslands were similar to those of younger woodlands. For older woodlands (>50 years), the amount of protected C was generally higher compared to remnant grasslands and younger woodlands. For all wooded areas irrespective of age, amounts of unprotected C were greater relative to amounts in remnant grasslands (Fig. 19).

C and N Sequestration Rates

Concentrations of C and N (g kg^{-1} soil) were converted to C and N stocks (g C m^{-2}) using previously determined soil bulk densities. Regression equations were fit to the data, and the slopes of the regression lines are the accumulation rates reported in Tables 8 and 9 for C and N, respectively. Rates of whole-soil C in the upper 15 cm of the profile averaged 10-30 g C m^{-2} yr^{-1} (Table 8). Most of the SOC appeared to be a consequence of higher rates of accrual associated with the free POM fraction (Table 8). This non-protected fraction accounted for 40 to nearly 100% of the whole- soil C accumulation rate. Macroaggregates also play an important role in C-sequestration as C accumulation within macroaggregates accounted for 30-70% of total SOC. Although the microaggregate fraction and all of its component parts appeared to be contributing much less to the overall rate of whole-soil C sequestration, this fraction still potentially accounted for 10-40% of total SOC. Intra-aggregate silt+clay may be an important component in aggregate-sized fractions, especially in drainages, as 60 and 90% of C accumulation in macro- and microaggregate-sized fractions in drainages, respectively,

Table 8. Soil organic carbon accumulation rates from linear correlations between woody stand ages and carbon stocks of soil fractions in cluster, grove, and drainage landscape elements at LaCopita Research Area. Numbers in parentheses are the standard errors of the estimates.

C Accumulation Rates (g C m^{-2} yr^{-1})			
	Cluster	Grove	Drainage
	0-15 cm	0-15 cm	0-15 cm
Whole Soil	10.5 (4.9)	14.2 (3.2)	29.3 (5.7)
Free POM	12.7 (4.5)	12 (3.2)	11.1 (4.7)
density<1.0g cm^{-3}	5.5 (2.5)	7.4 (2.2)	4 (3.0)
density<1.85g cm^{-3} (>250μm)	6.5 (2.0)	3.6 (1.2)	4.9 (1.9)
density<1.85g cm^{-3} (53-250μm)	0.7 (0.4)	1.1 (0.2)	2.2 (0.4)
Macro	5.3 (1.4)	10.1 (2.5)	7.9 (2.4)
MciPOM	1.5 (0.4)	0.5 (0.1)	1.1 (0.3)
MfiPOM	0.6 (0.1)	0.2 (0.05)	0.9 (0.1)
MiS+C	1.5 (0.4)	0.6 (0.1)	4.5 (1.0)
Micro	1.4 (1.4)	5.7 (1.5)	6.5 (1.4)
mfiPOM	0.2 (0.3)	-0.1 (0.2)	0.7 (0.4)
miS+C	0.3 (0.4)	-0.03 (0.4)	6 (1.3)
Free S+C	-1.2 (0.4)	-0.7 (0.3)	-0.5 (0.7)

Table 9. Soil nitrogen accumulation rates from linear correlations between woody stand ages and nitrogen stocks of soil fractions in cluster, grove, and drainage landscape elements at LaCopita Research Area. Numbers in parentheses are the standard errors of the estimates.

N Accumulation Rates (g N m^{-2} yr^{-1})			
	Cluster	Grove	Drainage
	0-15 cm	0-15 cm	0-15 cm
Whole Soil	0.8 (0.4)	1.1 (0.2)	2.6 (0.4)
Free POM	0.8 (0.2)	0.9 (0.2)	0.8 (0.3)
density < 1.0 g cm^{-3}	0.3 (0.1)	0.5 (0.1)	0.3 (0.2)
density < 1.85 g cm^{-3} (>250μm)	0.5 (0.1)	0.3 (0.07)	0.3 (0.1)
density < 1.85 g cm^{-3} (53-250μm)	0.05 (0.03)	0.1 (0.02)	0.2 (0.04)
Macro	0.4 (0.3)	0.1 (0.3)	0.7 (0.3)
MciPOM	0.07 (0.04)	0.01 (0.02)	0.1 (0.04)
MfiPOM	0.04 (0.01)	0.003 (0.02)	0.06 (0.02)
MiS+C	0.1 (0.03)	0.05 (0.03)	0.4 (0.1)
Micro	-0.02 (0.2)	-0.2 (0.1)	0.3 (0.3)
mfiPOM	-0.05 (0.01)	-0.03 (0.01)	-0.001 (0.04)
miS+C	0.03 (0.1)	-0.07 (0.07)	0.3 (0.1)
Free S+C	-0.2 (0.06)	-0.09 (0.03)	-0.1 (0.08)

was due to this occluded silt+clay fraction.

Rates of whole-soil N averaged 1-3 g N m^{-2} yr^{-1} (Table 9). As with SOC, much of the N accumulation (30-80%) in whole-soil was due to the higher rates of N sequestration in free POM (Table 9). The macroaggregate-sized fraction accounted for 5-30% of whole-soil N accumulation. Generally, the microaggregate-sized fraction and the free silt+clay fraction contributed little (<10%) to the overall rate of whole-soil N accumulation.

C:N Ratios of Whole Soil and Soil Fractions

C:N ratios of whole soil and soil fractions were not altered subsequent to woody plant invasion of grassland (data not shown). The C:N ratios of whole-soil and soil fractions were not statistically different among wooded landscape elements so values were averaged and compared to grassland values. Grassland and woodland C:N ratios were also not statistically different so values were averaged to obtain composite values for soil fractions (Fig. 20). The free light fraction, macro free POM, and macro-associated fractions (MciPOM, MfiPOM) had the highest C:N ratios (16-25). In contrast, microaggregates and microaggregate-associated fractions along with silt+clay fractions and whole soil had the lowest C:N ratios (10-13).

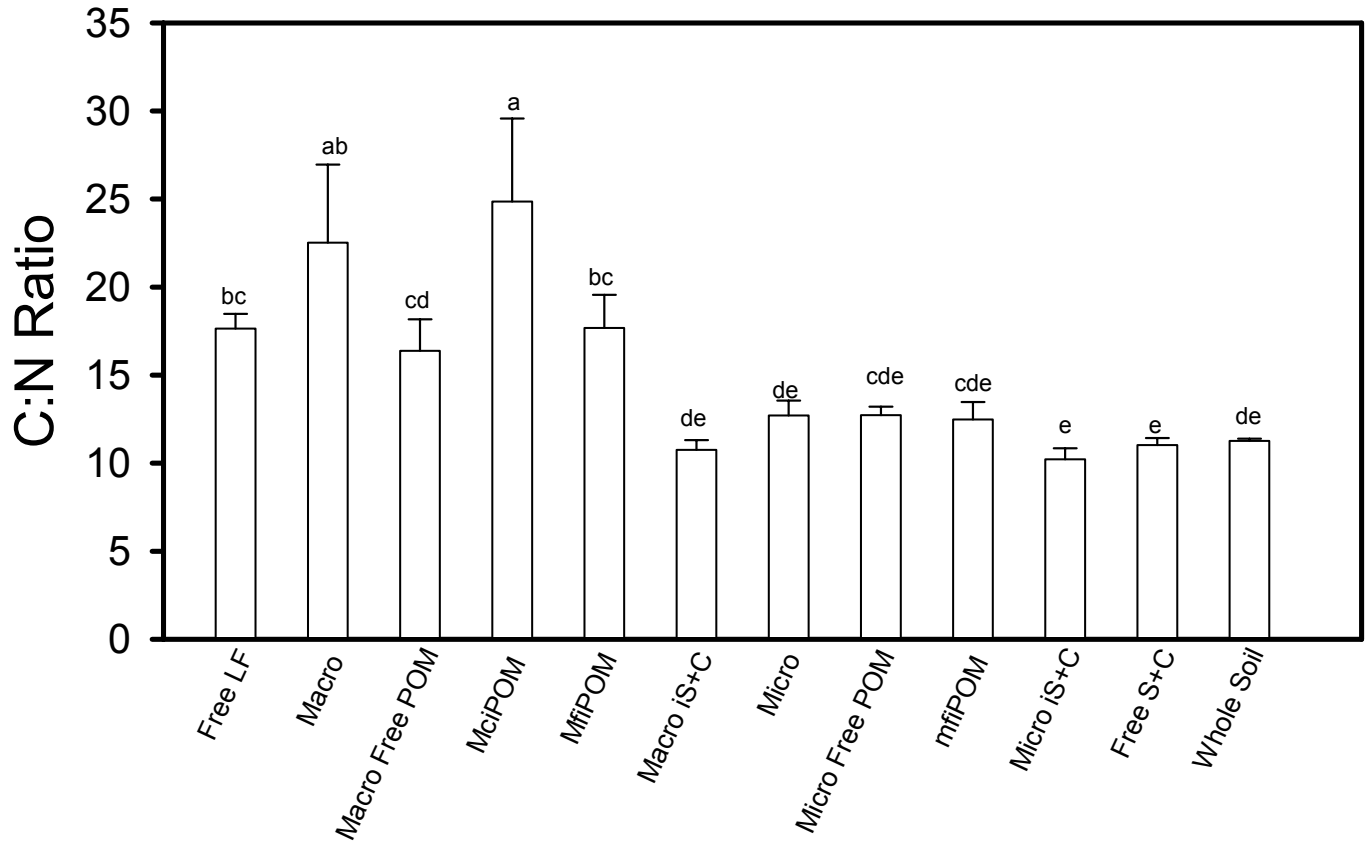

Fig. 20. Average C:N ratios of whole soil and soil fractions (0-15 cm) of remnant grassland and wooded landscape elements at LaCopita Research Area. Different letters indicate significant differences among soil fractions from Fisher's LSD following ANOVA..

DISCUSSION

Whole-soil C and N concentrations increased 100-500% following woody plant invasion of grassland (Fig. 15 and 16). In a system where low protection of organic matter is expected due to low silt+clay content, accumulation of organic matter results from increased sustained quantities of organic matter inputs to soil in excess of decomposition and/or inputs of residues with high chemical resistance (Baldock and Skjemstad, 2000). These whole-soil accumulations of C and N in woodlands are certainly a consequence of greater rates of primary production, surface litter pools, and root biomass in the wooded areas (Boutton et al., 1999; Archer et al., 2001; Hibbard et al., 2001). Organic matter inputs may also be of lower quality due to greater lignin concentrations, tannins, and other plant secondary compounds associated with woody plant species (Horner et al., 1988; Enríquez et al., 1993; Gillon et al., 1994; Hobbie, 1996; Köchy and Wilson, 1997; Kraus et al., 2003). Greater organic matter inputs to soil following woody plant proliferation in grasslands may also affect C and N sequestration by increasing the stabilization of the organic matter through protection afforded by increased aggregation. A direct relationship between aggregation and soil organic matter content has been observed in soils where organic materials are the dominant stabilizing agents (Oades and Waters, 1991).

Allocation patterns of C and N to aggregate fractions did not change with time following woody plant invasion of grasslands. Proportions of C and N within macro- and microaggregate-sized fractions were not significantly altered with grassland-to-woodland conversion. However, alterations to soil structure were evidenced by the

lower bulk densities in wooded areas relative to remnant grassland (Table 7) and by the changes in aggregate size distribution and aggregate-associated organic matter with time following woody plant invasion of grassland. Increases in free POM (external to aggregates) reflected the higher organic matter inputs from woody plants with time following woody plant encroachment. More stable macroaggregates were also formed following woody plant encroachment probably as a consequence of greater organic matter inputs. Once woody plants establish in grasslands, it appears that microaggregates are bound into stable macroaggregates, thus accounting for the increase in macroaggregates and the simultaneous decrease in microaggregates with time after woody invasion (Fig. 14). The decline in free silt and clay following woody invasion is also likely a consequence of their incorporation into macroaggregates. However, despite the increase in macroaggregates following woody plant invasion of grassland, most of the C and N (40-100%) is being accumulated in free POM that is external to aggregates and therefore not actually protected by stable soil structure. The dramatic increases in whole-soil C and N are probably being sustained by greater inputs and relatively slower turnover of POM, perhaps due to the biochemical recalcitrance of POM materials derived from woody vegetation or lack of sufficient nutrients and water for microbes to keep up with the additional inputs.

Although much of the C and N accumulation is in unprotected free POM fractions, it appears that the formation and stabilization of macroaggregates does play an important role in the protection and subsequent accumulation of organic matter following grassland-to-woodland conversion in this ecosystem. Woodlands contained a

greater proportion of water-stable macroaggregates than remnant grasslands. Macroaggregates potentially accounted for 30-70% of SOC accumulation rates and 5-30% of whole-soil N accumulation rates in woodlands. The greater formation of macroaggregates following grassland-to-woodland conversion may be facilitated or mediated by the significantly greater root biomass (Boutton et al., 1998, 1999; Archer et al., 2001; Hibbard et al., 2001) and soil microbial biomass (McCulley et al., 2004) in wooded landscape elements compared to remnant grassland. Macroaggregates are held together by temporary binding agents such as plant roots and fungal hyphae, and by transient agents (e.g. polysaccharides) (Elliott, 1986; Oades and Waters, 1991). Because of the labile nature of the organic binding agents, macroaggregates are considered less stable than microaggregates that are bound together mainly by persistent agents (aromatic humic materials) (Tisdall and Oades, 1982).

The wider C:N ratio (23) of macroaggregates in this study (Fig. 20) lends support to the idea that macroaggregates are held together by labile binding agents. Oades et al. (1987) suggested that organic matter with C:N ratios close to 20 are composed of plant materials in the early stages of decomposition. Buyanovsky et al. (1994) found that macroaggregates had wider C:N ratios than microaggregates, attributable to incompletely humified organic material, suggesting that active binding agents are root hairs and fungi. In contrast, they found narrower C:N ratios in microaggregates, indicative of organic matter that was highly processed and more persistent (longer mean residence time) (Buyanovsky et al., 1994). Clay fractions also had narrower C:N ratios (10) (Oades and Waters, 1991). Other studies have indicated that the organic matter

associated with macroaggregates and POM is less processed than that associated with microaggregates and silt and clay (Elliott, 1986; Oades et al., 1987; Beare et al., 1994; García-Oliva et al., 1994).

In this ecosystem, C and N concentrations in macroaggregates represent an important contribution to whole-soil C and N sequestration although this sink may not be long-term. Loss of POM associated with macroaggregates with cultivation indicates that the organic matter associated with macroaggregates is not stabilized (Beare et al., 1994). Others have shown that macroaggregates are sensitive to changes in soil organic matter as they are dependent on live binding agents (Tisdall and Oades, 1979). Therefore, disturbance or any changes in land-use practices may cause rapid destruction of stable macroaggregates and a decrease in associated soil C and N pools, particularly POM.

Turnover rates of organic matter in macroaggregates have been found to be variable ranging from a few years to decades (Skjemstad et al., 1990; Jastrow et al., 1996; Puget et al., 2000). It is generally agreed that microaggregates have considerably greater capacity to protect organic matter than macroaggregates (Krull et al., 2003). In this study, microaggregates accounted for approximately 50% of the total amount of protected C (C within aggregates and associated with silt+clay). The shift from grassland to woodland has resulted in a shift in fractions responsible for C-protection. In grasslands, microaggregates and associations with free silt+clay accounted for most of the protected C. In woodlands, microaggregates and macroaggregates account for about 85% of protected C. Macroaggregates may yet play a significant role in long-term organic matter storage and dynamics in this ecosystem since recent evidence suggests

that microaggregates form within stable macroaggregates. While macroaggregates may not afford long-term protection of organic matter, they may slow rates of decomposition of initial inputs and facilitate formation of micros within macros (Gale et al., 2000; Six et al., 2002b).

Although microaggregates are not sequestering as much C and N as the Free POM and macroaggregate-sized fractions, they still account for 10-40% of whole-soil C accumulation. Therefore, stabilization of C and N within microaggregates may constitute an important long-term sink for soil C and N sequestration in this ecosystem since the microaggregate sized fraction represented 60-80% of whole-soil by weight and may have longer turnover times as suggested by narrower C:N ratios. Microaggregates are considered to be more stable than macroaggregates since they are dominated by microbial products (persistent binding agents) (Oades and Waters, 1991).

It has been suggested that soils may have a finite capacity to protect organic matter and that the maximum protective capacity is related to the maximum amount of organic matter that can be associated with silt and clay particles (Hassink, 1997). Franzluebbers and Arshad (1996, 1997) showed that physical protection of organic matter within aggregates also increases with increasing clay content. Although relative proportions of protected C were lower in woodlands relative to remnant grassland, in terms of absolute amounts of C protected, woodlands had greater amounts relative to remnant grasslands (Fig. 19). The significant accumulations of C and N in this subtropical savanna parkland over the past century despite the low silt+clay content of the soils suggest that these soils have not reached a maximum storage capacity.

Additionally, the results of this study also suggest that the increased stability of macroaggregates appears to be a function of the increased plant production and organic matter inputs to soil. Boix-Fayos et al. (2001) indicated that stabilization of macroaggregates was a function of SOC content. The results of this study indicate that these areas undergoing woody plant invasion have been and may continue to be strong C and N sinks.

CONCLUSIONS

Whole-soil C and N have increased 100-500% following 130 years of woodland development. Woody plant invasion of grassland resulted in increased formation of water-stable macroaggregates, likely as a consequence of greater above- and belowground productivity, which increased organic matter inputs to the soil. The increase in soil structural stability was evident in decreased soil bulk density with time following woody invasion. Although aggregation increased following grassland-to-woodland succession, accumulation of C and N in the unprotected Free POM fraction accounted for most of the whole-soil C and N accumulation. This accumulation is likely due to the biochemical recalcitrance of woodland litter. Macroaggregates also accrued substantial amounts of C and N suggesting that aggregation is important in the protection and subsequent accumulation of soil organic matter. The greater accumulation of C and N in macroaggregates relative to microaggregates is probably due to the incorporation of microaggregates and silt+clay into macroaggregate structure. Although microaggregates were not responsible for much of the increase in C and N following woody plant

invasion (< 10%), microaggregates constitute an important long-term C and N sink due to their longer turnover times. The organic matter associated with microaggregates is considered more stable than that associated with macroaggregates. If microaggregates are bound into macroaggregates as recent studies suggest, then stable macroaggregates may contain an appreciable amount of C and N that is stablilized within microaggregates. The results of this study suggest that grassland ecosystems undergoing woodland development may be potentially large sinks for atmospheric CO_2 given the widespread geographical extent of this change in vegetation structure.

CHAPTER VI

ORGANIC MATTER SEQUESTRATION AND TURNOVER FOLLOWING WOODY PLANT INVASION OF GRASSLAND: EVIDENCE FROM NATURAL ^{13}C AND ^{15}N

INTRODUCTION

The level of organic C in soils is the net effect of organic matter inputs to soil and losses through decomposition (Schlesinger, 1997; Amundson, 2001). Because of the structural complexity and the heterogeneous nature of soil organic matter, rates of turnover vary along a continuum from labile organic materials that decay rapidly to more recalcitrant materials that remain in the soil for potentially thousands of years (Agren and Bosatta, 1996; Cadisch and Giller, 1997; Hassink et al., 1997; Baldock and Skjemstad, 2000). Soil organic matter dynamics and storage are affected by the physical form of the organic materials, the chemistry of the inputs, and the position they occupy within the soil matrix (Oades, 1988; Jastrow and Miller 1998). In particular, the role of soil structure in organic matter stabilization is of great importance because even labile organic materials may be adsorbed to clay surfaces or protected from decomposer organisms by incorporation into aggregate structure resulting in long-term storage of soil organic matter (Tisdall and Oades, 1982; Ladd et al., 1993).

Physical fractionation of soil organic matter has revealed that soil size and density separates differ in chemistry, rates of turnover, and dynamics (Tiessen and Stewart, 1983; Schulten, 1996; Golchin et al. 1997; Puget et al., 2000; Chefetz et al.

2002). For example, greater organic matter concentrations and higher mineralization rates are often reported to be associated with macroaggregate fractions. Conversely, organic matter associated with microaggregates may be more protected physically and more recalcitrant biochemically (Jastrow, 1996; Six et al., 2000). Because the location of organic matter within the soil structure is related to organic matter storage and dynamics, physical fractionation methods have been utilized to identify mechanisms of changes in C and N pools associated with land-use/land-cover changes (Elliott, 1986; Jastrow, 1996; Christensen, 2001; Six et al., 2002a; Jolivet et al., 2003).

Land uses and land cover changes have been shown to have significant impacts on soil physical structure that often result in changes in soil organic matter storage and turnover (Jastrow, 1996; Six et al., 2002a,b; Conant et al., 2003). One of the most geographically extensive land cover changes occurring around the world today is woody plant invasion of grass-dominated ecosystems, most likely due to livestock grazing and fire suppression (Jackson et al., 2000; Van Auken, 2000; Archer et al., 2001). Since grass-dominated ecosystems cover approximately 40% of the terrestrial surface and store more than 30% of global soil organic carbon (SOC), alterations to ecosystem structure and function due to woody encroachment could have significant consequences for ecosystem biogeochemistry and potential implications for global C and N cycles and climate (Schlesinger et al., 1990; Schlesinger, 1997). Many studies have shown that woody encroachment results in increased carbon storage in soils (Mordelet et al., 1993; Archer et al., 2001, 2004; Jessup et al., 2003). Current extrapolations and model results suggest that woody encroachment into grasslands and savannas may result in the

sequestration of 0.10 to 0.13 Pg C yr^{-1} in the USA, which represents approximately 20-40% of the current USA carbon sink strength (Tilman et al., 2000; Houghton et al., 2000, Pacala et al., 2001). Nonetheless, these regional estimates have a high degree of uncertainty, and some ecosystem-level studies have suggested that woody encroachment may result in no change (McCarron et al., 2003) or even net loss (Jackson et al., 2002) of soil carbon. Additional studies are needed to quantify the direction, magnitude, and mechanisms of change in soil carbon storage following woody encroachment into grass-dominated ecosystems.

In the Rio Grande Plains of southern Texas, grasslands dominated by C_4 grasses have undergone succession to subtropical thorn woodlands dominated by C_3 trees and shrubs, resulting in increased soil C and N storage (Boutton et al., 1998, 1999; Archer et al., 2001, 2004; Hibbard et al., 2001; McCulley et al., 2004). This shift in photosynthetic pathway provides a unique opportunity to utilize the natural abundance of ^{13}C and ^{15}N to evaluate changes in C and N cycles following woody plant invasion of grassland. Differences in $^{13}C/^{12}C$ ratios of plants utilizing the C_3 and C_4 photosynthetic pathways provides a natural tracer when a C_4 community type ($\delta^{13}C \approx -14$ ‰) is replaced by a C_3 community ($\delta^{13}C \approx -27$ ‰) or vice versa, permitting the quantification of the loss rate of C derived from the original vegetation and the simultaneous accumulation of new C derived from the current vegetation (Balesdent et al., 1988; Balesdent and Mariotti, 1996). Mean residence times (MRTs) of soil organic matter are a function of the rate at which $\delta^{13}C$ values of soil change over time following the change

in plant cover. Relatively few estimates of MRTs exist for soil organic matter under natural plant communities relative to agricultural systems (Paul et al., 1997).

Soil $\delta^{15}N$ values reflect the net effect of N-cycling processes as influenced by the biotic and abiotic environment (Robinson, 2001; Dawson et al., 2002). Globally, soil $\delta^{15}N$ values are positively correlated with mean annual temperature and negatively correlated with mean annual precipitation (Amundson et al., 2003). At the ecosystem level, soil $\delta^{15}N$ values are also influenced by a number of factors such as quantity and quality of organic matter inputs, soil N sources, and isotopic fractionation resulting from N-transformations (Nadelhoffer and Fry, 1988; Piccolo et al., 1994). In whole soil, $\delta^{15}N$ is related to degree of organic matter humification, increasing with higher degree of decomposition (Shearer et al., 1978; Turner et al.,1983; Nadelhoffer et al., 1996; Koba et al., 1998). However, few studies have evaluated patterns of $\delta^{15}N$ values in soil physical fractions. Kramer et al. (2003) found that $\delta^{15}N$ values of separated density fractions increased with increasing aliphaticity, which reflects greater microbial processing. Other studies have also shown that microbial processing results in ^{15}N accumulation (Adams and Grierson, 2001) although mechanisms of N fractionation associated with organic matter decomposition are still unclear. Because $\delta^{15}N$ tends to become enriched with increasing humification, $\delta^{15}N$ values of soil fractions may be related to MRTs and may be a potential indicator of soil organic matter quality.

The purpose of this study was to evaluate mechanisms of C and N storage and changes in C and N dynamics in relation to soil physical structure following woody plant invasion of grassland. To accomplish this, I utilized the natural abundance of ^{13}C and

^{15}N to 1) quantify amounts of C derived from C_4 vs. C_3 sources; 2) estimate the MRTs of the organic matter residing in physically isolated soil fractions; and 3) assess organic matter quality via the potential relationship between ^{15}N and MRTs of soil physical fractions.

MATERIALS AND METHODS

Study Area

Research was conducted at the Texas Agricultural Experiment Station La Copita Research Area (27° 40' N, 98° 12' W), located 65 km west of Corpus Christi, Texas, in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical, with a mean annual temperature of 22.4°C. Mean annual precipitation is 716 mm, with peaks in May-June and September. The topography consists of nearly level uplands which grade (1-3%) into lower-lying drainages and playas. Elevations range from 75 to 90 m. Domestic livestock have grazed this site over the past century.

Upland surface soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic horizon which contains non-argillic inclusions. C_4 grasslands interspersed with small, discrete clusters of woody plants dominate uplands. Species of *Paspalum, Bouteloua, Chloris,* and *Eragrostis* dominate in the grasslands. *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite) is the dominant plant species in all wooded landscape elements. Discrete clusters are characterized by a *Prosopis* overstory with species of *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory.

Where the argillic horizon is absent, clusters expand laterally and fuse to form larger groves of woody vegetation. Soils in lower-lying portions of the landscape are finer-textured clay loams (Pachic Argiustolls) and support closed-canopy woodlands. *Condalia hookeri* (M.C. Johnst.), *Celtis pallida* (Torr.), *Zanthoxylum fagara* (L.), *Diospyros texana* (Scheele.), and *Zizyphus obtusifolia* (T.& G.) dominate the understory beneath *Prosopis* in these lower-lying drainage woodlands. Evidence based on current vegetation patterns, sequential aerial photography, tree ring analysis, and the isotopic composition of soils all indicate conclusively that this entire landscape was once open C_4 grassland, and that woody plants have encroached into the grassland during the past 100 years (Archer et al. 1988, 2001; Boutton et al. 1998).

Chronosequence Approach

Soils were collected from the LaCopita Research Area using a chronosequence approach. Ten sites were sampled within remnant grassland, cluster, and grove upland landscape elements. In lower-lying drainage woodlands, 11 sites were sampled. All sites were located within an area of approximately 2 km^2. Soils from remnant grasslands represented time zero (i.e. prior to woody invasion), whereas soils from woody clusters, groves, and drainage woodlands were sampled in stands of known age.

Because the formation of cluster, grove, and drainage woodland landscape elements is initiated only after the establishment of *P. glandulosa* in grassland (Archer et al. 1988), the age of a woody plant stand corresponds to the age of the largest (presumably oldest) *P. glandulosa* in that stand. Ages of *P. glandulosa* were determined by measuring their basal diameters, and then converting those values to tree ages based

on regression equations specific to each landscape element (Stoker, 1997). In this study, woody plant stands were selected to encompass the full range of *P. glandulosa* basal diameters, corresponding to tree ages ranging from 10-130 years.

Collection of Soil Samples

Four soil cores (5-cm diameter x 30-cm depth) were taken beneath the largest *P. glandulosa* at each site, one in each cardinal direction from the bole. Sampling in remnant grassland sites was identical to woodlands with the exception that cores were taken in each cardinal direction from the base of a perennial C_4 grass plant. Prior to coring, soil surface litter was gently brushed aside to expose mineral soil. Soil cores were sectioned into 0-15 cm and 15-30 cm increments and stored at 4^{o}C. In the lab, each soil sample was thoroughly mixed and a subsample dried at 105^{o}C to determine bulk density. Then, the 4 cores from each site were pooled by depth increment and mixed. Only data for the 0-15 cm depth increment is reported in this study.

Soil Physical and Chemical Characterization

An aliquot of soil was passed through a 2-mm sieve to remove large organic matter fragments and used for physical, chemical, and isotopic analyses. Soil pH was determined on a 1:2 (soil:water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipette method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was dried at 60^{o}C, pulverized in a centrifugal mill, and used for elemental and isotopic analyses.

Soil Physical Fractionation

An aliquot of field-moist soils was first passed through a 15-mm sieve followed by an 8-mm sieve to remove large roots. Soils were then thoroughly mixed and air-dried. Using the disruptive forces of shaking and wet-sieving, soils were physically fractionated (Fig. 21) into 2 size classes of water-stable aggregates. Aggregates were further dispersed into POM and mineral components by shaking in sodium hexametaphosphate (HMP) by following a procedure modified from Elliott (1986) and Six et al. (1998). After oven-drying overnight at 65^{o}C, a subsample of soil from each site was immersed in deionized water on top of a 250-μm sieve. Organic debris and root pieces that floated in the water (density < 1.0 g cm^{-3}) were aspirated onto a 20-μm filter. This material was quantified as the free light fraction (Free LF) and considered to be the litter component of whole-soil.

After 5 minutes of slaking, the sieve was moved manually up and down 3 cm, 50 times over a 2 minute period. The fraction remaining on the 250-μm sieve was collected in a pre-weighed aluminum pan and oven-dried at 65^{o}C. Water plus soil <250 μm was poured through a 53-μm sieve and the sieving procedure was repeated. Unaggregated (free) silt and clay that passed through the 53-μm sieve was collected via centrifugation. This procedure yielded a macroaggregate-sized fraction >250 μm (Macro), a microaggregate-sized fraction 53-250 μm (Micro), and a free silt+clay fraction <53 μm (Free S+C). Silt and clay were not quantified separately because of the small percentage of silt and clay relative to whole soil at all sites.

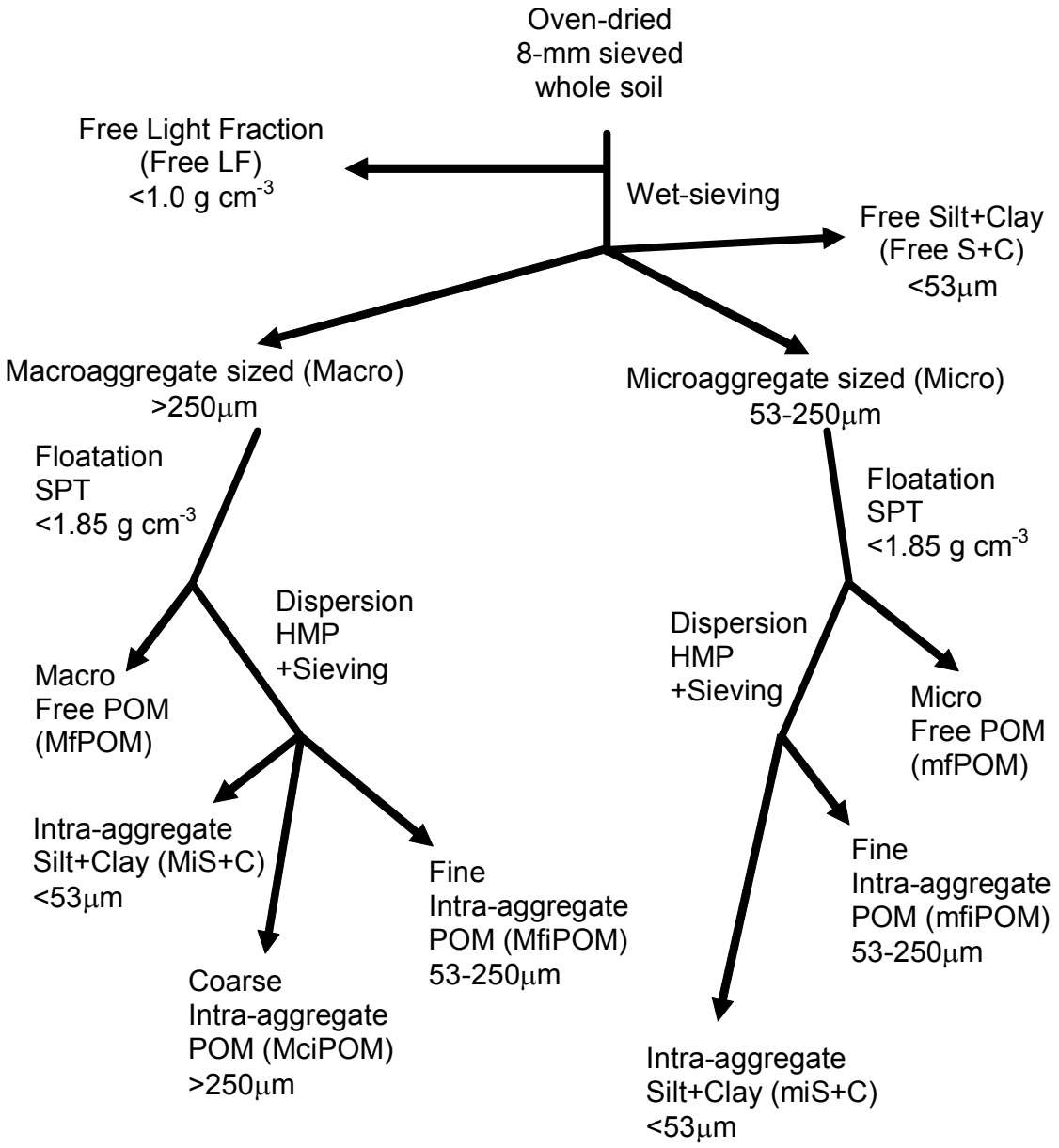

Fig. 21. Schematic for separation of soil organic matter into different size and density fractions (adapted from Elliot, 1986 and Six et al., 1998). POM= particulate organic matter, SPT=sodium polytungstate, HMP=sodium hexametaphosphate.

Both aggregate-sized fractions consisted of intact aggregates, sand of the same size class, and inter-aggregate POM of the same size class released during slaking and sieving. Inter-aggregate POM was separated from intact aggregates and sand by flotation of 5-g subsamples in sodium polytungstate (SPT) at a density of 1.85 g cm^{-3}. This material (Macro and Micro Free POM) was aspirated onto a 20-μm nylon filter, washed with deionized water, and dried. The remaining heavy fractions were washed with deionized water 3 times to thoroughly remove SPT. The heavy fractions were dispersed by shaking for 16 hours on a reciprocating shaker at 180 oscillations per minute in 5 g L^{-1} of HMP solution. Dispersed macroaggregates were passed sequentially through 250 and 53-μm sieves and rinsed with deionized water to yield coarse intra-aggregate POM >250 μm (MciPOM), fine intra-aggregate POM 53-250 μm (MfiPOM), and macroaggregated silt+clay (MiS+C). Dispersed microaggregates were passed through a 53-μm sieve to yield fine intra-aggregate POM 53-250 μm (mfiPOM) and microaggregated silt+clay <53 μm (miS+C). Intra-aggregate silt+clay <53 μm was collected after addition of 5.0 mL of 0.25M $CaCl_2$ + 0.25M $MgCl_2$ to flocculate clays.

Elemental and Isotopic Analyses

Soil fractions were analyzed for $\delta^{13}C$, $\delta^{15}N$, %C, and %N using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (ThermoFinnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Carbon and N isotope ratios are presented in δ notation:

$$\delta = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^3 \quad (4)$$

where R_{SAMPLE} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample and R_{STD} is the $^{13}C/^{12}C$ ratio of the V-PDB standard (Coplen, 1996) or $^{15}N/^{14}N$ ratio of atmospheric N_2 (Mariotti, 1983).. Precision of duplicate measurements was 0.1 ‰ for $\delta^{13}C$ and 0.15 ‰ for $\delta^{15}N$.

The relative proportions of SOC derived from the original C_4 grassland vegetation (F_C) vs. the more recent C_3 woodland vegetation was estimated by mass balance:

$$F_C = (\delta_T - \delta_{WL}) / (\delta_G - \delta_{WL}) \quad (5)$$

where δ_T is the $\delta^{13}C$ value of the SOC in some soil fraction at time T, δ_G is the average $\delta^{13}C$ value of SOC in that same fraction from remnant grasslands, and δ_{WL} is the average $\delta^{13}C$ value of organic matter inputs (roots, litter) in woodlands (-26 ‰).

Concentrations of free POM (density < 1.85 g cm^{-3}) were subtracted from macro- and microaggregate-sized fractions so that reported concentrations for these fractions only reflect organic matter contained within intact water-stable aggregates. Macroaggregate-sized fractions contained little to no sand >250 μm. Because microaggregate-sized fractions contained sand of the same size class as the microaggregates, all concentrations are reported on a whole-soil basis (g C or N in fraction kg^{-1} soil).

Statistical Analyses

ANOVA was used to test for statistical differences between soil chemical and physical characteristics attributable to landscape element (NCSS, 1995). To distinguish differences between measurements where ANOVA indicated significant effects due to landscape element, Fisher's LSD was used for mean separation. Fisher's LSD was also

used for mean separation for concentrations of protected C (POM within macro- and microaggregates and associated with silt+clay) and unprotected C (POM external to aggregates including MfPOM, mfPOM, and the Free LF fractions), proportion of protected C, and $\delta^{15}N$ values of soil fractions following ANOVA. Significance level was $p<0.05$. For changes in $\delta^{13}C$, non-linear regressions of the form $y = a+e^{-kt}$ were fit to the data using SigmaPlot 2001 (SPSS Inc., 2001). Non-linear regression equations of the form $y = e^{-kt}$ were fit to the F_C data vs. time; F_C was forced to be equal to 1.0 at time zero. R^2 values are not reported since they are not a legitimate descriptive statistic for nonlinear regression analyses (Neter et al., 1996).

RESULTS

Soil Chemical and Physical Characteristics

Particle size distribution in upland soils (grasslands, clusters, and groves) was approximately 80% sand, 10% silt, and 10% clay. In contrast, the particle size distribution of lower-lying drainage woodland soils was 60% sand, 20% silt, and 20% clay (Table 10). Soil pH was approximately 6.5 in remnant grasslands and was not altered following woody plant encroachment into grasslands (Table 10). Bulk density of the 0-15 cm depth interval decreased significantly from 1.2 g cm^{-3} in grasslands to 1.0 g cm^{-3} in wooded landscape elements (clusters, groves, and drainage woodlands).

Table 10. Comparison of soil chemical and physical characteristics from grassland and woodland landscape elements of a subtropical savanna parkland. Standard errors of the mean are in parentheses. Values within a row followed by the same letter are not significantly different at $p<0.05$.

	Grassland	**Cluster**	**Grove**	**Drainage**
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Sandy Loam
Sand (%)	80.5 (0.40)[a]	81.3 (0.5)[a]	81.4 (0.6)[a]	66.0 (3.0)[b]
Silt (%)	10.5 (2.0)[ab]	9.6 (1.6)[b]	12.0 (1.3)[ab]	15.4 (2.7)[a]
Clay (%)	9.0 (2.0)[b]	9.1 (1.6)[b]	6.6 (1.3)[b]	18.6 (1.9)[a]
pH	6.5 (0.1)[a]	6.4 (0.1)[a]	6.3 (0.1)[a]	6.2 (0.1)[a]
Bulk Density ($g\ cm^{-3}$)	1.2 (0.02)[a]	1.0 (0.03)[b]	1.0 (0.02)[b]	1.0 (0.04)[b]

Carbon and Nitrogen Concentrations (g C kg^{-1} soil) of Whole Soil and Soil Fractions

Whole soil C in the upper 15 cm of the profile increased on average by 80%-118% in upland clusters and groves and by 240% in drainage woodland landscape elements relative to remnant grasslands (Table 11). In woodlands, highest C concentrations were found in the unprotected free POM fraction, accounting for 34-53% of total SOC. In contrast, the unprotected free POM fraction of grasslands was only 20% of total SOC. In grasslands, macro- and microaggregates together constituted the largest proportion of total SOC (48-53%). Partitioning of C in macro- and microaggregates differed between grasslands and woodlands. In grasslands, microaggregates comprised the largest proportion of total SOC (44%). Microaggregates in woodlands ranged from 19-22% of total SOC. Macroaggregates were only 9% of total SOC in grasslands but ranged from 19-22% in wooded landscape elements. Within macro- and microaggregates, the intra-aggregate silt+clay fraction had the highest C concentration (15-50%) across all landscape elements. Free silt+clay was 25% of total SOC in remnant grasslands. In contrast, in woodlands, free silt+clay was only 8-10% of total SOC (Table 11).

The proportion of protected C (POM within aggregates and C associated with silt+clay) were greater than 60% for all landscape elements and was highest in grasslands (Fig.22). However, absolute concentrations of protected and unprotected C were higher in woodlands relative to remnant grasslands. The concentration of protected C increased 40-140% from 5 g C kg^{-1} soil in remnant grasslands to 7-12 g C kg^{-1} soil in woodlands. Concentrations of unprotected C increased 400-600% from 1 C kg^{-1} soil to

Table 11. Carbon and nitrogen concentrations (g kg^{-1} soil) of whole soil and soil size and density fractions from grassland, cluster, grove, and drainage woodland landscape elements of a subtropical savanna ecosystem. Standard errors are in parentheses.

	Grassland		**Cluster**		**Grove**		**Drainage**	
	C	N	C	N	C	N	C	N
Whole Soil	6.20 (0.28)	0.55 (0.02)	11.17 (1.31)	1.03 (0.10)	13.50 (1.58)	1.18 (0.12)	21.09 (3.07)	1.81 (0.27)
Free POM	1.21 (0.14)	0.07 (0.01)	5.91 (1.21)	0.37 (0.07)	7.09 (1.38)	0.50 (0.10)	7.25 (1.67)	0.51 (0.10)
density <1.0 g cm^{-3}	0.47 (0.04)	0.02 (0.001)	2.63 (0.60)	0.14 (0.03)	3.66 (0.91)	0.24 (0.06)	3.05 (0.93)	0.20 (0.07)
density <1.85 g cm^{-3} (>250µm)	0.53 (0.13)	0.03 (0.01)	2.57 (0.59)	0.17 (0.04)	2.58 (0.43)	0.19 (0.03)	3.37 (0.66)	0.23 (0.04)
density<1.85 g cm^{-3} (53-250µm)	0.21 (0.04)	0.02 (0.003)	0.72 (0.09)	0.05 (0.01)	0.85 (0.11)	0.07 (0.01)	1.00 (0.21)	0.08 (0.02)
Macroaggregate	0.57 (0.18)	0.05 (0.01)	2.48 (0.43)	0.19 (0.06)	3.01 (1.10)	0.20 (0.07)	4.00 (0.91)	0.36 (0.09)
MciPOM	0.14 (0.01)	0.01 (0.001)	0.48 (0.13)	0.03 (0.01)	0.37 (0.06)	0.03 (0.01)	0.55 (0.13)	0.05 (0.02)
MfiPOM	0.07 (0.01)	0.004 (0.001)	0.21 (0.05)	0.01 (0.003)	0.19 (0.02)	0.02 (0.004)	0.38 (0.08)	0.02 (0.01)
MiS+C	0.19 (0.04)	0.02 (0.004)	0.52 (0.13)	0.05 (0.01)	0.45 (0.07)	0.05 (0.01)	1.83 (0.44)	0.17 (0.04)
Microaggregate	2.70 (0.19)	0.25 (0.04)	3.39 (0.22)	0.30 (0.10)	3.50 (0.15)	0.34 (0.10)	6.44 (0.70)	0.47 (0.16)
mfiPOM	0.39 (0.02)	0.07 (0.001)	0.60 (0.07)	0.06 (0.002)	0.62 (0.04)	0.07 (0.002)	0.61 (0.12)	0.06 (0.01)
miS+C	1.10 (0.09)	0.12 (0.01)	1.34 (0.09)	0.16 (0.02)	1.50 (0.10)	0.17 (0.02)	3.23 (0.60)	0.28 (0.05)
Free Silt + Clay	1.53 (0.08)	0.15 (0.01)	1.09 (0.08)	0.11 (0.01)	1.13 (0.08)	0.11 (0.01)	1.93 (0.13)	0.16 (0.02)

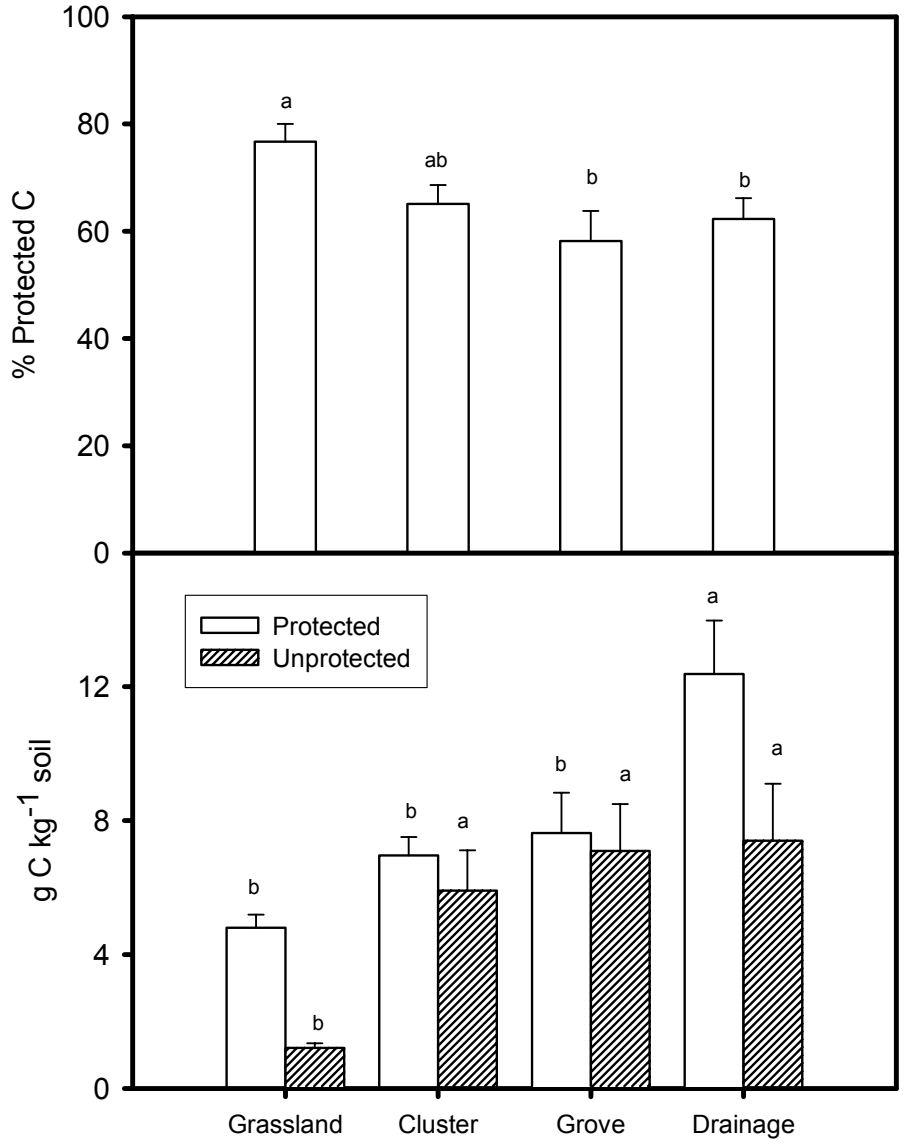

Fig. 22. Percent protected C (%) and C concentrations (g C kg^{-1} soil) of protected and unprotected soil C in grassland, cluster, grove, and drainage woodland landscape elements. Different letters above bars within a category (protected vs. unprotected) indicate significant differences among means from Fisher's LSD.

5-7 C kg^{-1} soil in wooded landscape elements (Fig. 22).

Similar to whole soil C, N concentrations in whole soil showed increases of 87-230% following woody plant invasion of grassland (Table 11). Nitrogen concentrations in soil fractions also paralleled C concentrations. The free POM fraction was only 13% of soil total N in grasslands but increased to 28-42% of soil total N in woodlands. Microaggregates in grasslands comprised the largest proportion of soil total N (45%). Microaggregates in woodlands ranged from 26-29% of whole-soil N. Macroaggregates were 10% of soil total N in grasslands and 17-20% of soil total N in wooded landscape elements. As with C concentrations, the intra-aggregate silt+clay fraction within macro- and microaggregates (MiS+C and miS+C) had the highest N concentration (25-60%) across all landscape elements. The free silt+clay fraction was 27% of total SOC in grasslands but only 9-11% of SOC in woodlands (Table 11).

Changes in the Natural Abundance of ^{13}C in Soil Physical Fractions

$\delta^{13}C$ values of all soil fractions in remnant grasslands ranged from approximately –16 to –20 ‰, typical of C derived from a C_4 grassland (Fig. 23). Following woody invasion, $\delta^{13}C$ values began to decrease in negative exponential fashion as grassland C decayed out of the system and woody plant-derived C accumulated. In all cases, the shifts in $\delta^{13}C$ followed exponential decay kinetics although the fractions differed in rates of change. The most rapid rates of isotopic change (steeper curves) were the free light fraction (density < 1.0 g cm^{-3}), Macro and Micro free POM (density < 1.85 g cm^{-3}), and macro coarse and fine iPOM fractions (MciPOM, MfiPOM), while the silt and clay fractions (MiS+C, miS+C, free S+C) showed slower rates of change (shallower curves).

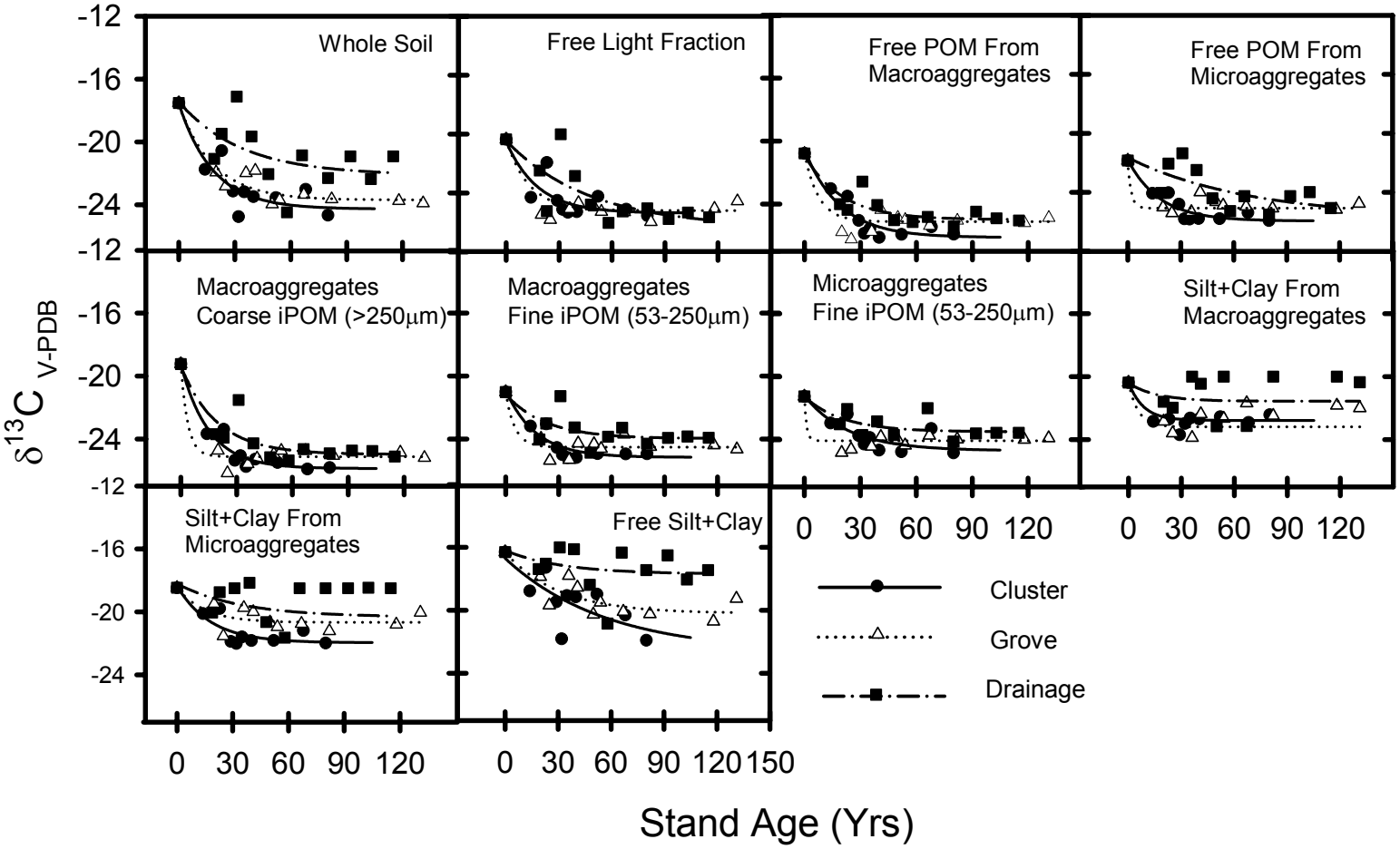

Fig. 23. Changes in $\delta^{13}C$ (‰ vs. V_{PDB}) values of whole soil and soil fractions with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at time zero and represent baseline starting values. The data were fit to an exponential decay model of the form $y = a + e^{-kt}$.

An isotopic mass balance model was utilized to compute F_C, the fraction of SOC derived from grassland (Eq. 5; Fig. 24). Fractional rate constants (*k*-values) for organic C decay were obtained by fitting exponential equations of the form $y = e^{-kt}$ to the F_C vs. time data in Fig. 23. Similar to changes in $\delta^{13}C$ values, the free light fraction and free POM fractions showed the most rapid decay of grassland-derived C out of the fractions whereas the silt+clay fractions indicated that 20-80% of grassland-derived C remained following 100 years of woody plant invasion.

Mean residence times (MRTs) were computed as the inverse ($1/k$) of the fractional rate constants (Table 12). The shortest MRTs (generally < 50 years) were associated with the unprotected free POM and macroaggregate-associated coarse and fine iPOM fractions. Macroaggregates were also relatively dynamic with MRTs ranging from 27-111 years. In contrast, microaggregates exhibited longer MRTs ranging from 71-476 years. Silt +clay (free and intra-aggregate) fractions had the longest MRTs (80-1111 years). In groves and drainage woodlands (but not clusters), the longest MRTs were associated with silt+clay within microaggregates. Whole soil MRTs ranged from 36-115 years, similar to the MRTs for macroaggregates. In addition to differences between soil fractions, there were also significant differences between landscape elements. More specifically, the longest MRTs were associated with drainage woodlands, which have higher silt+clay contents than the upland landscape elements.

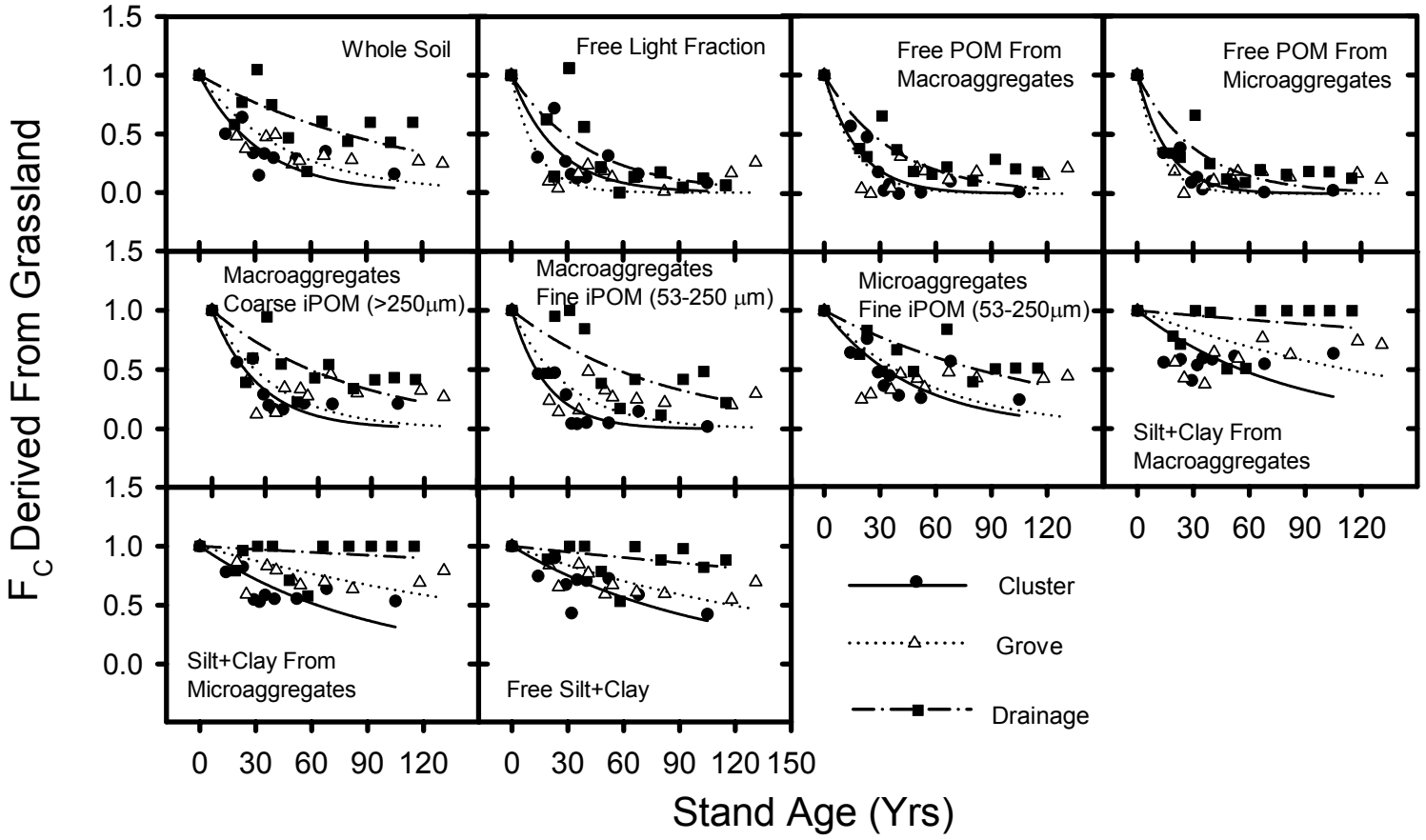

Fig. 24. The fraction of C derived from grassland (F_C) for whole soil and soil fractions with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at one, indicating that all soil C was derived from grassland. F_C was calculated by mass balance using the $\delta^{13}C$ value of whole soil and soil fractions from the original grassland and -26 ‰ as the $\delta^{13}C$ of woodland C.

Table 12. Rate constants (*k*) and mean residence times (MRT) of whole soil and soil size and density fractions from cluster, grove, and drainage woodland landscape elements of a subtropical savanna ecosystem. MRTs of macro- and microaggregates were calculated based on weighted averages of the C concentrations of the fractions comprising the aggregates. Standard errors are in parentheses.

	Cluster		Grove		Drainage	
	***k*-value**	**MRT (y)**	***k*-value**	**MRT (y)**	***k*-value**	**MRT (y)**
Whole Soil	0.0280 (0.0053)	36	0.0213 (0.0029)	47	0.0087 (0.0018)	115
Free POM	0.0479 (0.0078)	21	0.0673 (0.0204)	15	0.0239 (0.0047)	42
density <1.0 g cm^{-3}	0.0415 (0.0079)	24	0.0776 (0.0229)	13	0.0245 (0.0059)	41
density <1.85 g cm^{-3} (>250μm)	0.0547 (0.0074)	18	0.0619 (0.0210)	16	0.0269 (0.0041)	37
density <1.85 g cm^{-3} (53-250μm)	0.0523 (0.0094)	19	0.0336 (0.0078)	30	0.0123 (0.0028)	81
Macroaggregates	0.0369 (0.0043)	27	0.0402 (0.0117)	25	0.0090 (0.0021)	111
MciPOM	0.0630 (0.0058)	16	0.0865 (0.0257)	12	0.0317 (0.0050)	32
MfiPOM	0.0372 (0.0046)	27	0.0286 (0.0069)	35	0.0125 (0.0024)	80
MiS+C	0.0124 (0.0029)	80	0.0061 (0.0022)	162	0.0014 (0.0011)	714
Microaggregates	0.0141 (0.0023)	71	0.0084 (0.0020)	119	0.0021 (0.0008)	476
mfiPOM	0.0209 (0.0033)	48	0.0179 (0.0045)	56	0.0084 (0.0015)	119
miS+C	0.0110 (0.0018)	91	0.0045 (0.0009)	224	0.0009 (0.0007)	1111
Free Silt + Clay	0.0095 (0.0016)	105	0.0059 (0.0009)	170	0.0017 (0.0007)	589

Natural Abundance of ^{15}N in Soil Fractions

$\delta^{15}N$ values of soil fractions showed no relationship with time following woody plant encroachment into grassland (data not shown). $\delta^{15}N$ values of soil fractions were not significantly different between the three wooded landscape elements. Therefore, $\delta^{15}N$ values for soil fractions were averaged for cluster, grove, and drainage woodlands to obtain a composite woodland value for each soil fraction (Fig. 25). $\delta^{15}N$ values of roots, the free light fraction, macro free POM, macro coarse iPOM, macro iS+C, microaggregates, micro iS+C, and whole soil were less enriched in woodlands than remnant grasslands. In both grasslands and woodlands, surface litter and roots (see Chapter 2, p. 14-15 for collection methods) had the lowest $\delta^{15}N$ values (Fig. 25). All other fractions exhibited higher $\delta^{15}N$ values than litter and roots with the silt+clay fractions being the most enriched. Most POM fractions were not significantly different from each other with respect to $\delta^{15}N$ values for both grasslands and woodlands. Whole-soil $\delta^{15}N$ values of both grasslands and woodlands were not significantly different from macroaggregates.

A positive linear relationship was observed between $\delta^{15}N$ values of soil fractions and the MRTs derived from changes in ^{13}C dynamics (Fig. 26). Both $\delta^{15}N$ and MRTs increased in the sequence free light fraction < POM fractions < macroaggregates <microaggregates < S+C (free and intra-aggregate). Whole-soil values were intermediate between macro- and microaggregates in terms of $\delta^{15}N$ values and MRTs.

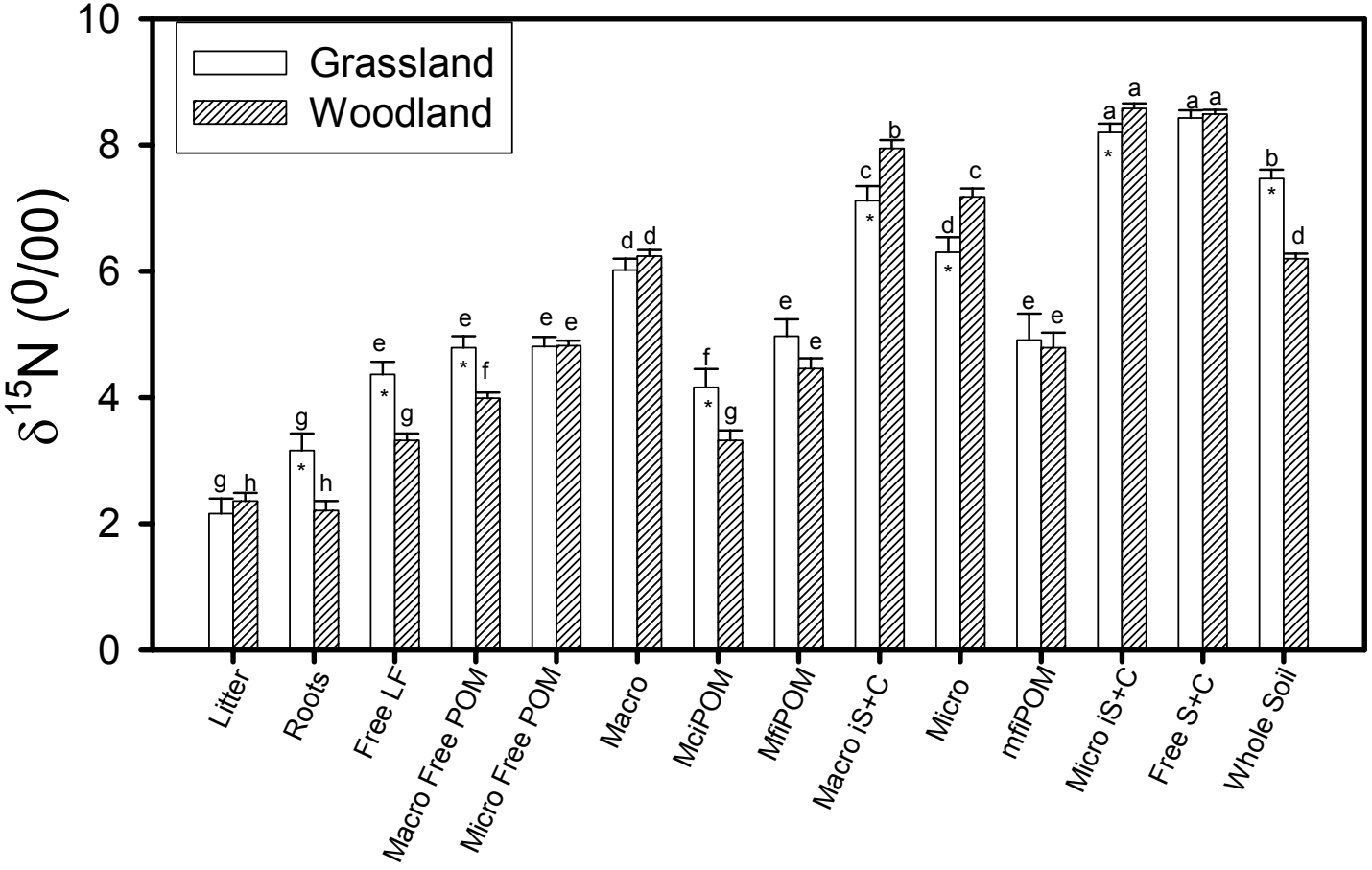

Fig. 25. $\delta^{15}N$ (‰) values of whole soil, litter, roots, and soil fractions (0-15 cm) from remnant grassland and woodland landscape elements at LaCopita Research Area. Woodland values are the mean of cluster, grove,and drainage woodlands. Asterisks indicate significant differences between grassland and woodland values ($p<0.05$). Different letters within grasslands and woodlands respectively indicate significant differences ($p<0.05$) among fractions.

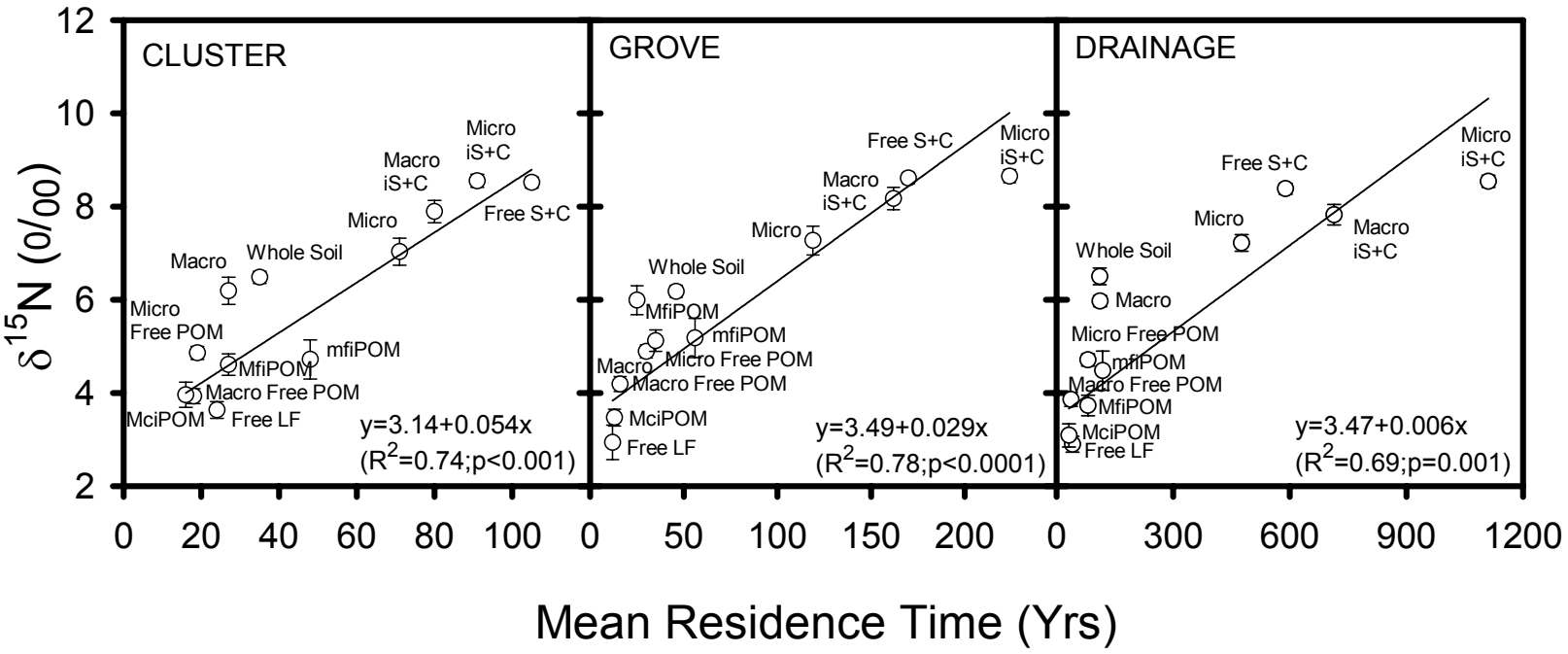

Fig. 26. $\delta^{15}N$ (‰) values of soil fractions versus mean residence times of the fractions derived from $\delta^{13}C$ dynamics for cluster, grove, and drainage woodland landscape elements.

DISCUSSION

Whole-soil C and N concentrations increased 80-240 % following woody plant invasion of grasslands (Table 11). These increases are within the range of whole-soil increases in C and N previously estimated in this subtropical savanna ecosystem and in other dryland ecosystems experiencing woody encroachment (Archer et al., 2001; Hibbard et al., 2001, 2003; Tiedemann and Klemmedson, 1973; Rundel et al., 1982; Virginia and Jarrell, 1983; East and Felker, 1993; Geesing et al., 2000). Increases in whole-soil C and N may be in part a consequence of the greater above- and belowground productivity of woodlands relative to remnant grasslands. Aboveground NPP increased from 1.9-3.4 Mg ha^{-1} yr^{-1} in remnant grasslands to 5.1-6.0 Mg ha^{-1} yr^{-1} in woodlands (Archer et al., 2001). Belowground productivity was also accelerated significantly as increases of up to 1000% for coarse and fine root biomass was found in woodlands compared with the original grassland (Hibbard et al., 2001).

Although whole-soil increases in C and N in this subtropical savanna ecosystem have been well-quantified, mechanisms of organic matter storage and rates of soil organic matter turnover have not been described. Concentrations of C and N in all soil fractions were greater in woodlands relative to remnant grasslands suggesting that the potential for C and N storage in these fractions is not currently saturated (Table 11). However, the highest C and N concentrations were found in the unprotected free POM fraction. Because the organic matter in this fraction is not protected within stable aggregate structure nor protected by association with silt and clay particles, accumulations of C and N in this fraction are probably being sustained by greater

organic matter inputs and by decreased decomposition, perhaps due to the poorer quality of woody plant inputs. The chemical composition of shrub leaf litter is considered to be more resistant to decay due to the increased presence of lignins, tannins, and other secondary compounds that are not present in herbaceous grassland litter (Horner et al., 1988; Enríquez et al., 1993; Gillon et al., 1994; Sharma et al., 1995; Hobbie, 1996; Köchy and Wilson, 1997; Kraus et al., 2003). Thus, slower decomposition rates due to lower quality substrates coupled with higher organic matter inputs may be responsible for the dramatic accumulations in C and N following woody plant invasion of grassland.

Nevertheless, physical protection of organic matter by stable soil structure and association with silt and clay may be contributing to SOC and total N storage in this dryland ecosystem. Protected C in this study was more than 60% of whole-soil C for all landscape elements (Fig. 22). Protected C in woodlands was greater than remnant grasslands due mainly to storage in macro- and microaggregates, which together accounted for 50% of whole-soil C and N. Less than 10% of total SOC and N were stored in the free silt+clay fraction (Table 11).

The increased fine and coarse root biomass and higher microbial biomass following woody plant invasion of grassland in this ecosystem (Boutton et al., 1998; Hibbard et al., 2001; McCulley et al., 2004) have likely fostered increased aggregation of soils in wooded areas. Following woody plant invasion of grassland, there was an increase in the proportion of macroaggregates in wooded landscape elements and concurrent decreases in proportions of free microaggregates and free silt+clay, likely reflecting their incorporation into macroaggregates (See Chapter 5., p.98). Greater root

biomass (coarse and fine) and associated rhizosphere activity in woodland soils likely promoted the formation of macroaggregates since roots and fungal hyphae are important binding agents operating at this scale (Tisdall, 1991; Jastrow and Miller, 1998). Bird et al. (2002) found that aggregate stability was higher beneath mesquite than in between mesquite canopies due to higher litter inputs, roots, and microbial activity. They also found higher concentrations of glomalin, a glycoprotein produced by arbuscular mycorrhizal fungal hyphae, beneath mesquite. Glomalin appears to be highly correlated with soil aggregate stability (Wright et al., 1999; Bird et al., 2002). Greater soil microbial biomass in this system also suggests that glomalin and other residues of microbial activity may be contributing to the stabilization of smaller macroaggregates and microaggregates (Tisdall, 1991).

Separation of soil organic matter into physical fractions in conjunction with the use of the natural abundance of ^{13}C and ^{15}N helps elucidate mechanisms of soil organic matter accumulation and turnover following grassland-to-woodland succession. $\delta^{13}C$ values of whole soil and soil fractions at time zero (i.e. prior to woody plant encroachment) ranged from -16 to -20 ‰, indicating that organic matter inputs were primarily of C_4 origin (Fig. 23). Following woody plant invasion, all soil fractions showed an exponential decline in $\delta^{13}C$ values reflecting the switch in organic matter inputs from C_4-dominated grasslands to C_3-dominated woodlands. These changes in $\delta^{13}C$ values were most rapid in the free light and POM fractions and slowest in silt+clay fractions.

The F_C data showed that following 100 years of woodland development, 10-30% of C derived from grasslands remained in whole soil (Fig. 24). This remaining C fraction appears to be relatively resistant to decay and is likely an important component of long-term C storage. Proportion of C_4-derived C in the free light fraction and POM fractions declined rapidly following woody plant invasion indicating rapid turnover in these fractions. Unprotected organic matter fractions are often relatively labile with fairly high concentrations of carbohydrates and nitrogen compounds (Skjemstad et al., 1996; Golchin et al., 1994, 1995). Because the organic matter in these fractions are not protected within aggregates or associated with clay minerals, they are readily accessible to microbes as reflected by their initial rapid loss following woody plant encroachment. Thus, initial decomposition of this unprotected organic matter fraction is a direct function of its chemical composition. With increasing degree of decomposition, organic matter may be transferred to more stabilized soil fractions. In contrast to the rapid decomposition of C_4-derived C from unprotected soil fractions, a substantial amount of C_4-derived C remained in silt+clay fractions reflecting slower turnover rates. The long-term protection of soil organic matter by silts and clays has been well-established (Anderson and Paul, 1984; Ladd et al., 1985; Feller and Beare, 1997; Hassink, 1997). Silts and clays are often associated with strongly humified organic residues of high molecular weight that are resistant to decomposition. However, clays may also react with labile organic matter constituents resulting in longer turnover of those otherwise labile fractions.

Radiocarbon dating of silts and clays indicate the importance of organo-mineral complexing in protection of organic matter from decomposition resulting in long turnover times (Anderson and Paul, 1984). Anderson and Paul (1984) found MRTs ranging from 170 years for fine clay-associated organic C, 800-965 years for coarse and fine silt, and 1255 years for coarse clay. Balesdent et al. (1988) found that MRT for silt was 400 years and 1000 years for clay. Monreal et al. (1997) found that the mineral fraction (< 50 μm) was comprised of resistant organic C that was highly humified with a turnover time of 275 years (Monreal et al., 1997). The average MRT of 361 years for silt+clay fractions in this study is a combination of the different turnover times associated with silt and clay size fractions and is a long-term pool for C storage.

The MRTs of soil fractions derived from ^{13}C dynamics corroborate that free POM fractions have faster turnover rates (mean = 26 years) than aggregate-protected (mean = 47 years) and silt+clay associated fractions (mean = 361 years) (Table 12). MRTs calculated from natural ^{13}C dynamics following conversion of native forest to cultivation were 12 years for free POM (external to macro- and microaggregates) and 33 years for occluded POM (within aggregates), reflecting the decreased turnover of organic matter protected within stable soil structure (Besnard et al., 1996). Light fraction or free POM is comprised of younger C (i.e. more recently added residues) that contains recognizable plant material (Gregorich et al., 1995; Puget et al., 2000; Baisden et al., 2002). Average turnover for macroaggregates (54 years) was faster than turnover for microaggregates (222 years) (Table 12). MRTs for Macro fine iPOM (MfiPOM) was longer than MRTs for Macro coarse iPOM (MciPOM) and free POM fractions

suggesting that this fraction is more protected by aggregate structure. Some of the MfiPOM is likely POM within microaggregates that have been incorporated or formed within the macroaggregate structure, hence the longer MRTs of the MfiPOM fraction relative to MciPOM. However, MfiPOM had faster turnover rates than Micro fine iPOM (mfiPOM), indicating that microaggregates have a greater protective capacity than macroaggregates, likely due to small pore spaces and slower diffusion of oxygen into microaggregates (Sexstone, 1985). Faster turnover of MfiPOM may also be indicative of new microaggregates being formed within macroaggregates whereas free microaggregates reflect more stabilized material nearing the end of the microaggregate turnover cycle.

Buyanovsky et al. (1994) found that macroaggregates had approximately the same life span as vegetative fragments reflecting a labile C pool. Unlike macroaggregates that are held together by temporary, labile binding agents (roots and fungal hyphae), microaggregates are held together by microbial polysaccharides and more persistent binding agents (aromatic humic material) that have slower turnover (Tisdall and Oades, 1982; Elliott and Coleman, 1988). Monreal et al. (1997) found via chemical characterization that the rapid turnover of organic matter in macroaggregates was due to the presence of plant residues and high activity of soil microorganisms. Microaggregates had intermediate kinetic and molecular properties intermediate between macroaggregates and silt+clay and had an average MRT of 61 years (Monreal et al., 1997). Other studies indicate that turnover of macroaggregates is accelerated relative to microaggregates although rates are extremely variable ranging from a few years for

macroaggregates to a century or more for microaggregates (Skjemstad et al., 1990; Buyanovsky et al., 1994; Jastrow et al., 1996; Monreal et al., 1997; Puget et al., 2000). The results of this and other studies all confirm that turnover of organic matter appears to be dependent on its position within soil structure.

However, the long MRTs of the silt+clay fractions in this study irrespective of location within or exterior to soil macro- or microaggregates suggests that soil texture plays an important role in C and N accumulation somewhat independent of soil structure. Evidence for additional protection of silt+clay-protected organic matter is reflected in the fact that microaggregate-associated silt+clay in groves and drainages were longer than any other silt+clay fraction. Generally though, MRTs of silt+clay fractions in aggregates were variable and not consistently longer than estimated MRTs of free silt+clay external to aggregates. It appears that protection of organic matter via associations with silts and clays have long MRTs regardless of position within or external to aggregates. The longer MRTs of soil fractions from drainage woodlands is also likely due to the protective effects of soil texture since this lower-lying portion of the landscape contained higher concentrations of silt+clay relative to uplands. Longer MRTs of POM fractions in drainages may be due to coating with mineral matter. Longer MRTs may also be due to reductions in the diffusion of oxygen into finer-textured soils. Prior studies at LaCopita Research Area indicated that drainage woodland soils retained the greatest memory of the original C_4 grassland in the clay fraction reflecting the slow turnover associated with clay-associated organic matter (Boutton et al., 1998; Boutton et al., 1999). The range of MRTs across soil fractions in

this study is consistent with the idea of a range of organic matter fractions that differ in dynamics and turnover.

Turnover of soil organic matter gives an indirect assessment of organic matter quality. Longer turnover times are associated with older, more resistant C pools. Use of the natural abundance of ^{15}N in soil fractions may help elucidate information concerning the quality of organic matter that has accumulated following grassland-to-woodland succession. Globally, whole-soil $\delta^{15}N$ values are positively correlated with mean annual temperature and negatively correlated with mean annual precipitation (Amundson et al., 2003). At the ecosystem level however, controls on soil $\delta^{15}N$ values are uncertain and appear to be influenced by a number of factors such as quantity and quality of organic matter inputs, soil N sources, and isotopic fractionation resulting from N-transformations (Nadelhoffer and Fry, 1988; Piccolo et al., 1994). Despite the uncertainty in the interpretation of $\delta^{15}N$ values, there are some generalities. Plant and litter inputs are generally less enriched in ^{15}N relative to soil due to plant uptake of mineral N which is isotopically depleted compared to organic N (Gebauer and Schulze, 1991; Handley and Raven, 1992). $\delta^{15}N$ values increase with increasing microbial transformation of residues in litter inputs since microbial discrimination of ^{15}N occurs during N-catabolic processes in the soil (Andreux et al., 1990; Christensen, 1992). Successive microbial decomposition of N-containing substrates results in the progressive increase in ^{15}N of soil organic matter.

Several studies have shown increasing $\delta^{15}N$ values with decreasing particle size (Ledgard et al., 1984; Tiessen et al., 1984; Kerley and Jarvis, 1997) although few studies

have evaluated patterns of $\delta^{15}N$ values in soil physical fractions. Baisden et al. (2002) showed increasing $\delta^{15}N$ values with increasing density. Heavier density organic matter fractions are associated with increasing degree of organic matter decomposition (Golchin et al., 1995). Kramer et al. (2003) found that increased humification due to greater microbial processing was correlated with increases in $\delta^{15}N$ values of soil organic matter fractions. The results of this study are in agreement with Kramer et al. (2003). Lower $\delta^{15}N$ values were associated with recent organic matter inputs (litter, roots, POM fractions) (Fig. 25). At the other extreme, higher $\delta^{15}N$ values in silts+clays indicate that the organic matter in these fractions is older and more humified. Results from a correlated study at LaCopita Research Area showed increasing $\delta^{15}N$ values with increasing lignin degradation in soil fractions, consistent with the notion that $\delta^{15}N$ values become more enriched as decomposition proceeds (Gamblin et al., 2003).

Because $\delta^{15}N$ tends to become enriched with increasing humification, $\delta^{15}N$ values of soil fractions may be related to MRTs of the same fractions and thereby provide additional information about soil organic matter quality. MRTs were positively correlated to ^{15}N values of soil physical fractions suggesting that fractions with higher 15N values and longer MRTs are more humified (Fig. 26). Longer MRTs and more enriched $\delta^{15}N$ values in microaggregates and in silt+clay fractions relative to macroaggregates and free POM fractions which had shorter MRTs and lower $\delta^{15}N$ values suggests that the organic matter associated with microaggregates and silt+clay fractions may be not only physically protected, but also biochemically recalcitrant.

CONCLUSIONS

Woody plant invasion of grasslands in this subtropical savanna ecosystem in southern Texas has resulted in increased SOC and soil total N pools. Increased C and N pools in soils are due to both the retention of older C_4-derived organic matter by protection within microaggregates and association with silt+clay and the accumulation of new C_3-derived organic matter in macroaggregates and POM fractions. The MRTs of the soil fractions derived from ^{13}C dynamics indicated that the C and N associated with microaggregate and silt+clay fractions were in a form that promoted longer turnover times. $\delta^{15}N$ values of soil fractions indicated that the organic matter associated with macroaggregates and POM fractions is of recent origin and the organic matter associated with microaggregates and silt+clay is more humified. Although much of the C and N in woodlands are stored in free POM with an average turnover of 26 years, a significant proportion is also stored in aggregates with MRTs of up to 400 years suggesting that these areas experiencing woody plant proliferation are behaving as relatively long-term C and N sinks. Soil texture also appears to play an important role in organic matter sequestration independent of soil structure since organic matter associated with silts and clays had long turnover times regardless of position within or external to soil aggregates. Use of the natural abundance of ^{13}C and ^{15}N in evaluating the relationship between soil organic matter and soil structure helps provide mechanistic explanations for alterations to C and N processes and dynamics following grassland-to-woodland succession.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Human land-use practices (livestock grazing and fire suppression) have caused increased woody plant abundance in many grass-dominated ecosystems worldwide (Archer et al., 2001; Van Auken, 2000). Whether affected ecosystems are a net source or sink of atmospheric CO_2 remains controversial. In the Rio Grande Plains of southern Texas, highly productive woody plant species have encroached into open grasslands over the past 150 years. This shift in vegetation structure has altered C and N cycling in these affected ecosystems.

C and N storage in litter, roots, and soils increased significantly following woody plant invasion of grassland. Most of the accumulation of C and N in roots and soils occurred in the upper 15cm of the profile although smaller changes were observed at 15-30cm. Despite documented increases in soil respiration rates and net mineralization rates (Hibbard et al., 2001; McCulley et al.,2004), it appears that organic matter inputs have exceeded losses over the past century such that these areas in southern Texas undergoing grassland-to-woodland succession have been behaving as net C and N sinks over the past century.

Rates of accumulation in the total soil system (litter + roots + soil) ranged from 21-62 g C m^{-2} yr^{-1} and 2-5 g N m^{-2} yr^{-1}, respectively. Rates of C and N accumulation in soil comprised the largest proportion of accumulation in the total soil system, ranging from 12-43 g C m^{-2} yr^{-1} and 1-3 g N m^{-2} yr^{-1}, respectively. Rates of C and N accumulation in these three compartments have been linear for the past century, and

show no signs of departing from linearity at the present time. These accumulations of C and N in litter, roots, and soils are likely due to higher rates of net primary productivity in wooded landscape elements compared to remnant grasslands. Rates of soil C and N accumulation in this study were consistent with previous estimates from this same site based on field measurements (Archer et al., 2001, 2004) and from simulation modeling using CENTURY (Hibbard et al., 2003). These accumulation rates of soil C and N have caused whole-soil C and N pools to increase by 80-200% following woody plant invasion of grassland.

Several potential mechanisms help explain the accumulation of SOC and STN following woody plant invasion. Greater soil microbial biomass (SMB) was documented beneath woodland soils relative to remnant grasslands likely as a result of the increased organic matter inputs (above- and belowground) in woodlands. The SMB to SOC ratio (C_{mic}/C_{org}) suggests that the quality of the organic matter inputs in woodlands may be poorer than that of grasslands since less biomass is supported per unit SOC. Woody plant tissues generally have higher lignin and secondary compounds making them more resistant to decomposition (Horner et al., 1988; Enríquez et al., 1993; Gillon et al., 1994; Sharma et al., 1995; Hobbie, 1996; Köchy and Wilson, 1997; Kraus et al., 2003). In conjunction with lower C_{mic}/C_{org}, the higher metabolic quotient (qCO_2) in woodlands also suggests that organic matter quality may be poorer in woodlands since microbes are less efficient at converting available C into biomass. Thus, SOC and STN may be accumulating in soils due to decreased decomposition as a result of lower quality organic matter inputs.

However, size and density fractionation of soil revealed that aggregation has increased with time following woody plant invasion of grassland suggesting that organic C and N are potentially less available to microbes due to physical protection within aggregates. The protection of organic matter within soil aggregates is another plausible mechanism whereby SOC and N may be accumulating in soil irrespective of organic matter quality. Even if organic matter quality was not altered following woody plant invasion of grassland, protection within aggregates and association with silt and clay allows greater C and N sequestration in soil by physically isolating organic matter from microbial accessibility, thus decreasing decomposition (Ladd et al., 1993). The increases in SMB and root biomass likely promoted this increase in soil aggregation observed following woody plant invasion via the binding of microaggregates and silts and clays into larger macroaggregates.

Most of the whole-soil C and N accumulation rates were due to storage in free POM external to aggregates, corroborating the idea that higher amounts of woody inputs may also possess a degree of biochemical recalcitrance that results in slower turnover. C concentrations in this unprotected C pool increased 400-600% following woody plant invasion of grassland. This free POM that is external to aggregates is comprised of C_3-derived organic matter which likely includes roots and fungal debris. The wider C/N ratios of macroaggregates and POM indicate that these fractions are composed of relatively new organic matter that is less processed than that associated with microaggregates and silt+clay.

Mean residence times (MRTs) of soil fractions derived from ^{13}C dynamics indicate that silt+clay fractions have the slowest turnover times averaging 360 years whereas unprotected POM fractions have the fastest turnover (about 30 years). These MRTs coupled with $\delta^{15}N$ values of the same soil fractions suggest that the organic matter associated with microaggregates and silt+clay is also more rendered than that associated with fresh organic matter inputs (litter and roots) and macroaggregates and POM.

Although most of the C and N accumulated in free POM fractions, this fraction is a relatively slowly cycling pool of C as indicated by an average MRT of 30 years. Additionally, average turnover times of fine POM within aggregates was approximately 60 years, reflecting the importance of soil structure in organic matter protection. In fact, absolute concentrations of protected C (within macro- and microaggregates and associated with free silt+clay) were higher in woodlands by 40-140% relative to remnant grasslands. Accumulation of C and N in macro- and microaggregates collectively could potentially account for at least 50% of C and N accumulation in whole soil.

Collectively, the results of this study suggest that increases in SOC and STN have increased primarily due to higher organic matter inputs and slower turnover of the organic matter likely due to the poorer quality of woody litter relative to herbaceous grassland litter. However, protection of organic matter within aggregates and by association with silt and clay fractions is also an important mechanism contributing to the significant increases in soil C and N following woody plant invasion of grasslands. This study enhances current understanding of the effects of land uses (livestock

production) and land cover changes (grassland-to-woodland conversion) on mechanisms of soil C and N sequestration in rangelands. Because similar grassland-to-woodland conversions have been geographically extensive in grasslands and savannas worldwide, processes and mechanisms of soil C and N storage and dynamics documented here could have significance for understanding the global C and N cycles and the earth's climate system.

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VITA

NAME: Julia Den-Yue Liao

EDUCATION:

Ph.D. 2004	Rangeland Ecology	Texas A&M University	College Station, TX
M.S. 1996	Rangeland Ecology	Brigham Young University	Provo, UT
B.S. 1994	Conservation Biology	Brigham Young University	Provo, UT

Permanent Mailing Address: c/o Mr. and Mrs. Edward Liao. 1701 Aberdeen Dr., Arlington, TX 76015

AWARDS:

- DOE Graduate Fellowship, Global Change Education Program 2001-2004
- Outstanding Ph.D. Student, Department of Rangeland Ecology & Management, Texas A&M University, 2004
- George Bush Presidential Library Foundation Grant recipient for College of Agriculture and Life Sciences, Texas A&M University, 2003
- 1st place, graduate student poster competition, Agriculture Program Conference, Texas A&M University, 2001
- NASA Graduate Fellowship, Earth System Science Program, 2000-2001

INVITED PRESENTATIONS:

J.D. Liao, T.W. Boutton, and J.D. Jastrow. 2004. Soil organic matter dynamics following land-cover change in a subtropical savanna: Insights from soil physical fractionation and stable isotopes. AGU Fall Meeting, San Francisco, CA. Dec. 2004.

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Boutton, T.W., S.R. Archer, and J.D. Liao. 2002. Land cover changes and soil carbon dynamics: Insights from natural ^{13}C and long-term incubations. p.49-50. *In* Carbon cycle management in terrestrial ecosystems. Proc. 9th US/Japan Workshop on Global Change. US Global Change Research Program, Washington D.C.

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